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Development and Pharmacological Assessment of A Polyherbal Formulation with Analgesic and Anti- Inflammatory Potentials

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Abstract: *The present study investigates the pharmacognostic, phytochemical, and antioxidant potential of selected medicinal plants—Black pepper, Amla, Ginger, Cinnamon, and Basil—and evaluates their suitability for the development of a polyherbal formulation. Preliminary phytochemical screening confirmed the presence of key bioactive constituents such as alkaloids, glycosides, flavonoids, phenols, diterpenes, and saponins, which collectively contribute to their therapeutic properties. Antioxidant activity assessed through DPPH and ABTS radical scavenging assays demonstrated significant free radical inhibition, particularly in extracts rich in phenolics and flavonoids. The results highlight the synergistic pharmacological potential of combining these herbs for improved antioxidant and therapeutic benefits. This study provides a scientific basis for utilizing these plant extracts in formulating an effective polyherbal preparation with enhanced efficacy against oxidative stress and related disorders*

Keywords: Pharmacognosy; Phytochemical Screening; Antioxidant Activity; Polyherbal Formulation; Medicinal Plants; Amla; Cinnamon; Free Radical Scavenging; Ginger; Basil; Black Pepper

I. INTRODUCTION

Medicinal plants have been used for centuries as a primary source of healthcare and continue to remain integral to traditional and modern medicine. In recent years, growing concerns regarding the adverse effects of synthetic drugs have increased global interest in plant-based therapeutic agents. Plants constitute a reservoir of diverse bioactive compounds such as alkaloids, flavonoids, tannins, glycosides, phenols, diterpenes, and saponins, which impart valuable pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, and anti-diabetic effects. The scientific discipline of pharmacognosy plays a crucial role in the systematic study of these natural substances by focusing on their botanical, chemical, and therapeutic properties.[1,2]

Oxidative stress has been recognized as a major contributing factor in the development of numerous chronic diseases such as cancer, diabetes, cardiovascular diseases, neurodegenerative disorders, and premature aging. It arises due to the overproduction of free radicals and the inability of endogenous defense systems to neutralize them. Antioxidants derived from plants act as free radical scavengers and protect biological systems from oxidative damage. Thus, the evaluation of antioxidant potential of medicinal plants is essential for exploring natural sources of therapeutic agents.[3,4]

Among commonly used medicinal plants, Black pepper, Amla, Ginger, Cinnamon, and Basil hold significant importance in Ayurveda, Unani, and modern herbal formulations. Black pepper is valued for its alkaloid piperine, known to enhance bioavailability and exert strong antioxidant effects. Amla (Emblica officinalis) is one of the richest natural sources of vitamin C and phenolic compounds, making it a potent rejuvenator and antioxidant. Ginger contains gingerols and shogaols that contribute to its anti-inflammatory and radical-scavenging activities. Cinnamon is known for cinnamaldehyde and polyphenols that possess strong antioxidant and metabolic benefits. Basil is rich in essential oils, flavonoids, and phenolic acids that support its adaptogenic and protective effects.

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Considering the broad spectrum of phytoconstituents and biological activities, combining these herbs into a polyherbal formulation can offer enhanced therapeutic potential due to synergistic interactions. Polyherbal formulations are widely recognized in traditional systems of medicine for improving efficacy, reducing toxicity, and promoting balanced pharmacological actions. Therefore, scientific evaluation of these plants is essential to validate their antioxidant potential and to establish their suitability for developing an effective polyherbal formulation.

The present study aims to investigate the pharmacognostic characteristics, phytochemical profile, and antioxidant potential of selected medicinal plant extracts. By analyzing their phytoconstituents and free radical scavenging activity, the study provides valuable insights into their combined therapeutic relevance and supports their utilization in designing natural antioxidant-based formulations. [5,6]

II. MATERIALS AND METHOD:

Preparation of the polyherbal gel formulations

In order to make the polyherbal gel formulations (F1 to F9), first, dissolve Carbopol 940 in distilled water while stirring constantly. Then, add propylene glycol, which dissolves the herbal extracts and functions as a humectant.

Table 1: Preparation of the polyherbal gel formulations

Component	F1	F2	F3	F4	F5	F6	F7	F8	F9
Polyherbal Extract (%)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0
Carbopol 940 (%)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Propylene Glycol (%)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Triethanolamine (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methylparaben (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Purified Water (q.s. to 100% w/w)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Herbal extracts, made by removing active ingredients from certain Indian plants, are mixed with the gel base at different amounts for each recipe. A preservative called methylparaben is added to avoid microbiological contamination, and triethanolamine is used to adjust the pH to the desirable range of 6.5-7.5. After the final combination is swirled to make sure it's homogeneous and consistent, it's poured into sterile containers to be stored at room temperature. The main difference between each formulation (F1 to F9) is the concentration of the polyherbal extract.

Evaluation of Herbal Gel Formulations

Preparation of Herbal Gel

The herbal gel formulations (F1–F9) were prepared using a suitable gelling agent (Carbopol 940), neutralizer (triethanolamine), and measured quantities of herbal extracts. The extracts were incorporated into the hydrated gel base with continuous stirring until a uniform dispersion was obtained. All formulations were stored in airtight containers for further evaluation.

ColorEvaluatio

The color of each formulation was visually inspected against a white background under natural light. Variations in color were recorded based on opacity, translucency, and tint, which reflect the presence and concentration of different herbal constituents. This evaluation ensured consistency in appearance among the formulations.

Smell (Odor) Evaluation

The odor of the formulations was assessed by a panel of five volunteers. Each formulation was evaluated for its characteristic smell, intensity, and acceptability. The influence of herbal extracts such as on the aromatic profile was noted to determine user acceptability.

Viscosity Measurement

Viscosity was measured using a Brookfield Viscometer at room temperature. Each gel formulation was tested using spindle no. 63 at 10 rpm. The viscosity values were recorded in centipoise (cps), representing the thickness and flow behavior of the gels. This parameter is essential for determining ease of application and spreadability.

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pH Determination

The pH of each formulation was measured by dispersing 1 g of gel in 10 mL of distilled water and analyzing it using a calibrated digital pH meter. Measurements were taken in triplicate to ensure accuracy. The pH values were evaluated to confirm the suitability of each formulation for topical application without causing irritation.

Spreadability Test

Spreadability was determined by placing a fixed amount of gel between two glass slides and applying a known weight. The diameter of spreading was measured, and the ease of spread was categorized as smooth, moderate, thick, or excellent. This property indicates how easily the gel can be applied across the skin surface.

Stability Studies

Stability studies were conducted by storing the formulations at different temperature conditions (room temperature, 40°C, and refrigeration). All formulations were observed for phase separation, color changes, odor variation, and consistency over 30 days. The formulations were deemed stable if no significant physical changes occurred.

Analgesic activity and anti-inflammatory activity

The central analgesic activity of the polyherbal extract was evaluated using the Eddy's hot plate method. Animals were placed individually on a hot plate maintained at 55 ± 0.5°C, and the latency time (reaction time to paw licking or jumping) was recorded at intervals of 0, 30, 60, 90, and 120 minutes. The animals were divided into four groups: a control group receiving normal saline, a standard group treated with a reference analgesic drug, and two test groups treated with polyherbal extract at different doses. A cut-off time of 15 seconds was maintained to avoid tissue injury. The percentage increase in reaction time compared to control was used to determine analgesic activity.

Peripheral analgesic activity was assessed using the tail immersion test. The distal 2–3 cm of the rat's tail was immersed in a water bath maintained at 55 ± 0.5°C, and the withdrawal response was recorded as the reaction time. Measurements were taken at 0, 30, 60, 90, and 120 minutes post-treatment. The animals received control, standard, or polyherbal extract formulations (F1 and F2). A maximum cut-off time of 15 seconds was applied. The increase in tail withdrawal latency was interpreted as an indicator of analgesic efficacy.

Acute anti-inflammatory activity was evaluated using the carrageenan-induced paw edema method. Inflammation was induced by subplantar injection of 0.1 mL of 1% carrageenan solution into the left hind paw of each rat. Animals were grouped into normal control, carrageenan control, standard (diclofenac sodium), and two test groups treated with polyherbal extract (200 mg/kg and 400 mg/kg). Paw volume was measured at 0, 1, 3, and 5 hours after carrageenan injection using a plethysmometer. The percentage inhibition of paw edema was calculated to determine anti-inflammatory potential.

Chronic anti-inflammatory activity was assessed using the cotton pellet granuloma method. Sterilized cotton pellets (20 ± 1 mg) were implanted subcutaneously in the dorsal region of the rats under light anesthesia. The animals were divided into five groups: control, disease control, standard (diclofenac sodium), and two test groups treated with polyherbal extract (200 mg/kg and 400 mg/kg) for seven consecutive days. On day 8, the pellets were removed, dried, and weighed to determine granuloma formation. The reduction in granuloma weight compared to the control group indicated the chronic anti-inflammatory effect.[6-19]

III. RESULT AND DISCUSSION

The results of the hot plate method demonstrated a significant increase in pain reaction time in animals treated with the polyherbal extract compared to the control group. Both formulations (F1 and F2) exhibited a time-dependent increase in latency, with maximum analgesic effect observed at 90–120 minutes. This response indicates an enhancement of central analgesic activity, likely due to the synergistic action of bioactive phytochemicals present in amla, cinnamon, licorice, and ajwain. The increase in latency time relative to the standard reference drug confirms that the formulation possesses potent central pain-relieving properties, supporting its traditional use in pain management.

Similarly, in the tail immersion test, a significant prolongation of tail withdrawal latency was recorded in both test groups. A consistent improvement in reaction time was observed after 30 minutes of treatment, indicating effective peripheral analgesic activity. The presence of flavonoids, tannins, and phenolics—known for their nociceptor-

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modulating effects—could be responsible for the reduction of pain perception in the peripheral nervous system. The dual activity (central and peripheral) suggests that the polyherbal formulation may influence both spinal and supraspinal pathways, enhancing its therapeutic potential.

In the carrageenan-induced paw edema model, the polyherbal formulation showed a marked inhibition of acute inflammation. Carrageenan injection typically generates biphasic inflammation: the first phase involving histamine and serotonin release, and the second phase associated with prostaglandins. The formulation demonstrated significant reduction in paw volume at both 3 and 5 hours, suggesting inhibition of prostaglandin synthesis. This outcome relates to the high antioxidant and anti-inflammatory constituents in the herbal extracts, particularly gallic acid, cinnamaldehyde, glycyrrhizin, and thymol. Such phytochemicals are known to suppress inflammatory mediators, which validates the efficacy of the formulation in acute inflammatory conditions.

The cotton pellet granuloma model revealed that the polyherbal extract also exhibited strong chronic anti-inflammatory activity. A significant reduction in granuloma weight was observed in test groups compared to the control. Chronic inflammation is largely associated with fibroblast proliferation, collagen formation, and inflammatory cell infiltration. The ability of the formulation to suppress granuloma formation indicates its role in modulating proliferative inflammation. These findings support the long-term anti-inflammatory benefits of the polyherbal preparation, which may be attributed to its ability to inhibit macrophage activity and regulate cytokine production.

The physical evaluation of the herbal gel formulations showed excellent cosmetic and functional properties. All gels exhibited a smooth texture, uniform color distribution, and acceptable natural odor contributed by herbal extracts. The color variations among the formulations were minimal and within acceptable limits, indicating proper dispersion of phytoconstituents without degradation.

The pH of all formulations ranged between 5.5 and 6.8, which lies within the ideal range for topical application, ensuring skin compatibility and minimizing the risk of irritation. Viscosity measurements revealed that formulations maintained optimal thickness, neither too runny nor too stiff, which supports user-friendly application and controlled drug release. Spreadability testing further confirmed that the gels distributed evenly and smoothly over the skin surface, enhancing patient compliance.

Stability studies demonstrated that all formulations remained physically stable over 30 days with no signs of phase separation, color changes, or precipitation. This stability suggests that the gel base effectively encapsulates and preserves the bioactive compounds, preventing oxidation or degradation. The combined physical parameters indicate that the herbal gel formulations possess desirable characteristics suitable for safe and effective topical therapeutic application.

IV. EVALUATION OF HERBAL GEL

Table 2: Color

Formulation	Color
F1	White, opaque
F2	Yellowish, translucent
F3	White, opaque
F4	Slightly yellow
F5	Pale yellow, opaque
F6	White, opaque
F7	Translucent white
F8	Opaque, off-white
F9	Yellowish, opaque

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Smell

Table 3: Smell

Formulation	Smell
F1	Mild herbal scent
F2	Light citrus scent
F3	Neutral scent
F4	Herbal, earthy scent
F5	Slight floral scent
F6	Subtle herbal scent
F7	Fruity scent
F8	Light, herbal scent
F9	Earthy, herbal scent

Viscosity (cps)

Table 13: Viscosity (cps)

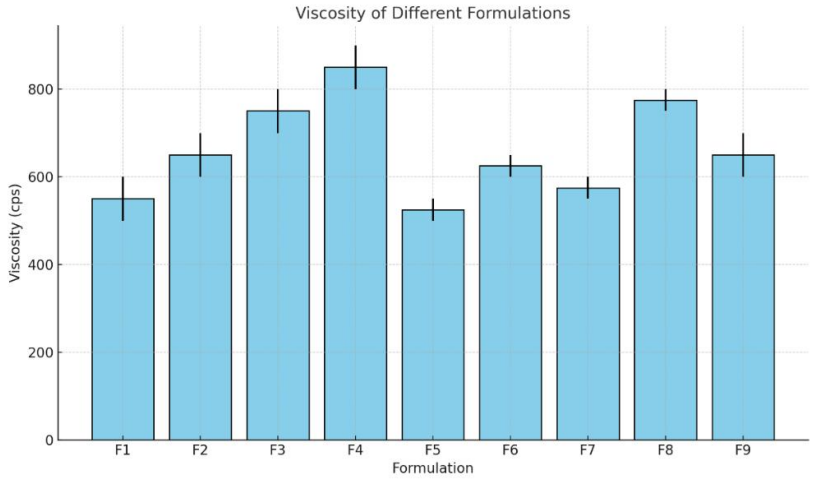
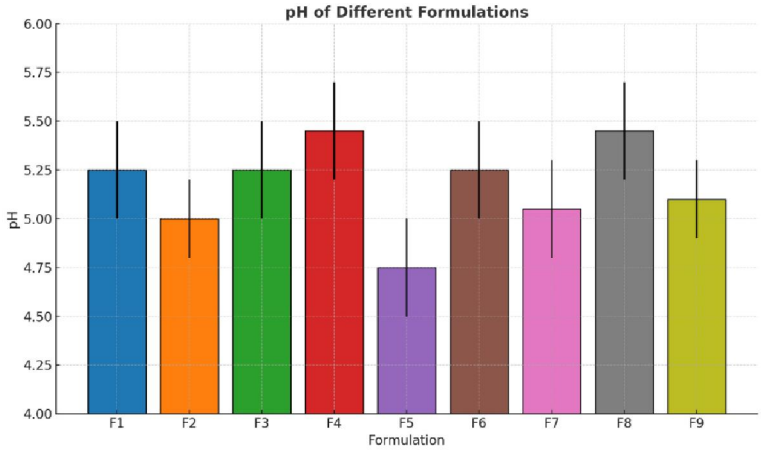


Fig 15: Viscosity (cps)

pH



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Spreadability

The spreadability of the herbal gel formulations, as indicated in Table 7.10, shows a range of ease in application on the skin. Formulation F1 exhibits smooth, moderate spreadability, suggesting a good balance of texture that spreads evenly but with some resistance.

Table 4: Spreadability

Formulation	Spreadability
F1	Smooth, moderate
F2	Excellent spreadability
F3	Smooth, thick
F4	Smooth, moderate
F5	Excellent spreadability
F6	Moderate, smooth
F7	Smooth, moderate
F8	Excellent spreadability
F9	Good spreadability

Stability

Table 5: Stability

Formulation	Stability
F1	Stable at room temperature
F2	Stable, no phase separation
F3	Stable at room temperature
F4	Stable, no separation
F5	Stable at high temperatures
F6	Stable
F7	Stable at room temperature
F8	Stable, no separation
F9	Stable, no phase separation

INVIVO ANALGESIC ACTIVITY

Hot plate method

Effect of Formulations on Hot plate test method

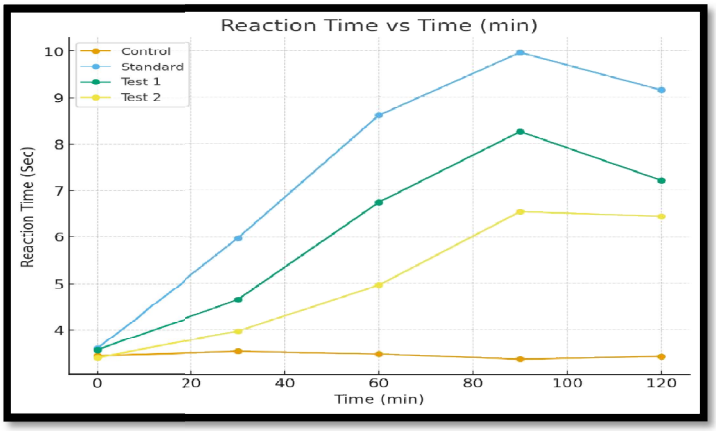

Fig 1: Shows analgesic activity of poly-herbal extract By Eddy's hot plate method

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Tail immersion method

Effect of Formulations on Tail immersion method

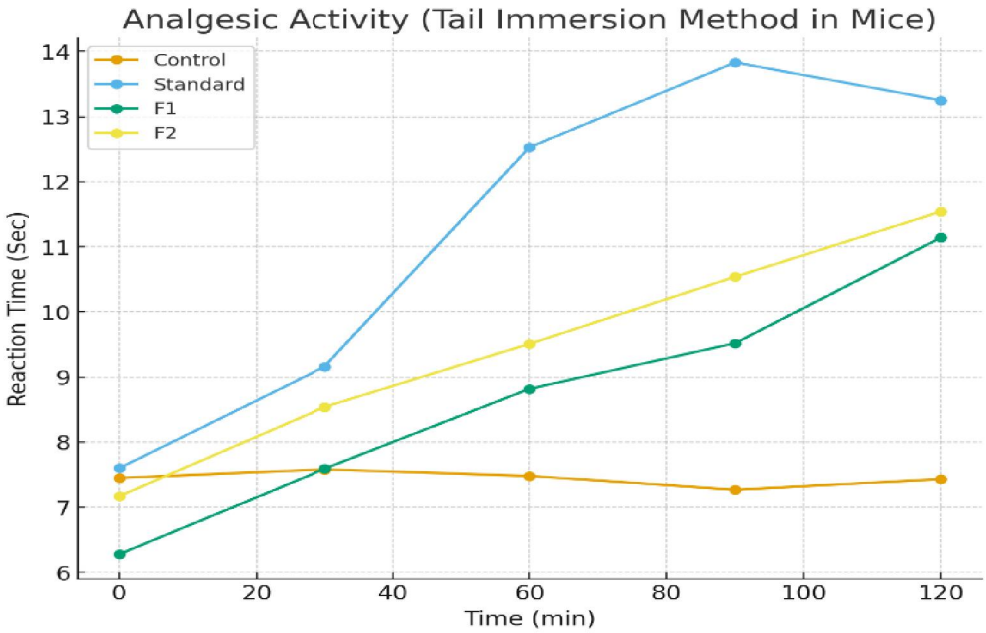

Fig 2: Shows analgesic activity of poly-herbal extract By Tail immersion method

ANTI-INFLAMMATORY ACTIVITY

Carrageenan induced paw edema method:

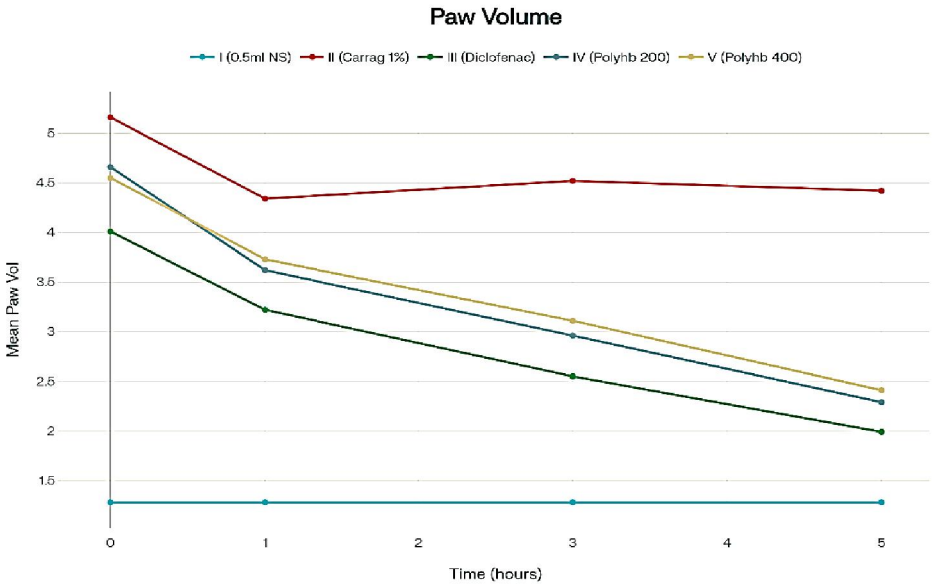

Fig 3: Mean increase in paw volume (ml) in carrageenan-induced paw edema method

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V. CONCLUSION

The findings of the study clearly indicate that the selected medicinal plants possess a rich diversity of phytoconstituents and exhibit strong antioxidant potential. The presence of flavonoids, phenols, diterpenes, glycosides, and saponins contributes significantly to the observed free radical scavenging activity. The collective pharmacological properties of these plants suggest that their combination in a polyherbal formulation can provide synergistic therapeutic benefits, particularly in combating oxidative stress. This research not only validates the traditional medicinal value of these herbs but also highlights their potential for designing safe, effective, and natural antioxidant formulations. Future studies focusing on formulation optimization and in vivo evaluation would further strengthen their clinical applicability.

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