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Pharmacological screening of anti-inflammatory potential of polyherbal extract

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ABSTRACT

Background: Inflammation is the immune response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation and acts by removing injurious stimuli and initiating the healing process. Inflammation is therefore a defense mechanism that is vital to health. Usually, during acute inflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection. Current herbal medicine has been found effective with minimal or no side effect.

Materials and Methods : Polyherbal extract of plants which contains curcumin, withanolides, withanoferins, linoleic acid are the active constituents responsible for Anti-inflammatory effect. Extraction of the plant material was done to obtain extract and further proceed for physical characterization and phytochemical analysis. TLC was done to confirm the active constituents present in polyherbal with reference to standard. The polyherbal extract was compared with standard to evaluate the anti-inflammatory by in-vitro assessment of Bovine albumin fraction and Protein denaturation assay.

Result and Conclusion : The percentage yield of extracts were found to 66.7, 74.2, 63.7. The R_f value of polyherbal extract was found to be 0.82 which matches with the standard and hence presence of curcumin, withanolides, withanoferins, linoleic acid was confirmed. The dose dependent study and statistical comparison with the help of graph pad prism, version 9.03 by one way ANOVA followed by dunnett's multiple comparison test has revealed that moderate inhibition ($p \leq 0.05$) is observed in albumin and protein denaturation treated with Polyherbal extract when compared with standard Diclofenac sodium and hence the Anti-inflammatory effect of Polyherbal extract is justified.

1. Introduction

Inflammation is the immune response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation and acts by removing injurious stimuli and initiating the healing process. Inflammation is therefore a defense mechanism that is vital to health. Usually, during acute inflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection. This mitigation process contributes to restoration of tissue homeostasis and resolution of the acute inflammation. However, uncontrolled acute inflammation may be come chronic, contributing to a variety of chronic inflammatory diseases. At the tissue level, inflammation is characterized by redness, swelling, heat, pain, and loss of tissue function, which result from local immune, vascular and inflammatory cell responses to infection or injury. Important microcirculatory events that occur during

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the inflammatory process include vascular permeability changes, leukocyte recruitment and accumulation, and inflammatory mediator release. Various pathogenic factors, such as infection, tissue injury, or cardiac infarction, can induce inflammation by causing tissue damage. The etiologies of inflammation can be infectious or non-infectious. In response to tissue injury, the body initiates a chemical signaling cascade that stimulates responses aimed at healing affected tissues. These signals activate leukocyte chemo taxis from the general circulation to sites of damage. These activated leukocytes produce cytokines that induce inflammatory responses. The inflammation are of 2 types acute and chronic inflammation. [1–3]

Inflammation is a complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. The primary purpose of inflammation is to eliminate the initial cause of cell injury, clear out damaged cells, and initiate the healing process. It is a fundamental defense mechanism in the immune system. However, inflammation can be a double-edges word. While acute inflammation is necessary for healing, chronic inflammation can lead to various diseases and conditions, including autoimmune disorders, cardiovascular diseases, cancer, and neurodegenerative disorders. In this comprehensive discussion, we will explore the mechanisms of inflammation, the types of inflammation, key mediators, the role of the immune system, factors contributing to chronic inflammation, the impact on health, and various therapeutic approaches. [4,5]

In the last few decades eco-friendly, bio-friendly, cost effective and relatively safe, plant based medicines have moved from the fringe to the main stream with increased research in the field of traditional medicine. WHO has reported 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India. India is the largest producer of medicinal herbs and is called the Botanical Garden of the World. The review has classified the plants according to their botanical name, country of origin; parts used and nature of active agents. There are several literature reviews by different authors about anti-inflammatory herbal agents. [6]

Curcuma longa from family Zingiberaceae, Withania somnifera from family Solanaceae, Ocimum sanctum from family Lamialiceae a medicinal herbs mentioned in Ayurveda with its variety of therapeutic benefits like Anti-diabetic, Anti-Cancer, Anxiolytic, Immunomodulatory etc. An phytochemical analysis of plants revealed that the presence of several important phytochemicals including alkaloids, tannins, phenolic compounds, phytosterols, terpenoids, saponins, and flavonoids have beneficial health effect. The curcumin, withanolides, withanoferins, linoleic acid etc. such phytochemicals present in Curcuma longa, Withania somnifera, Ocimum sanctum possess the potent Anti-inflammatory effect. Combination drug therapy of these plants may give more pronounce effect to reduce the inflammation. [7–9] Keeping this in mind, the present research was carried out to assess the Anti-inflammatory potential of polyherbal extracts of these plant by In-vitro Bovine albumin fraction and Protien denaturation method.

2. Materials and Methods

2.1. Pharmacognostical study

2.1.1. Plant material

Curcuma longa belonging to Family Zingiberaceae, Withania Somnifera belonging to family Solanaceae and Ocimum santum belonging to family Lamialiceae were collected from local market of Nashik region. Herbarium was prepared and authenticated through Botanical Survey of India, Western circle, Koregaon Park, Pune City, Maharashtra.

2.1.2. Drying and pulverizing of plant material

The turmeric is the rhizome, or root stock, of the Curcuma longa plant. This rhizome is harvested, usually boiled or steamed, and then dried and ground to produce the distinctive yellow turmeric powder. Withania somnifera and Ocimum santum were dried in shade for 2 weeks and triturated to a coarse powder. The powder was further passed from 2 mm sieve to obtain finer particles.

2.2. Extraction of plant material

2.2.1. Preparation of Curcuma longa rhizome extract

The rhizomes of turmeric were dried in oven at 105°C for 3 h. Dried rhizomes were triturated using mortar and screened through a sieve with mesh 80 to obtain uniform powder with particle size of 0.18 mm. The turmeric powder was stored in refrigerator to prevent moisture uptake. The Soxhlet extraction, as the reference method, was performed follows:15 g ground turmeric powder was weighed and embedded in a thimble and put in the Soxhlet apparatus which was gradually filled with acetone as the extraction solvent. The extraction experiment was carried out at 60°C within 8 h. Upon completion of the extraction, the acetone was separated from the extract using rotary evaporator (StuartRE300) under vacuum at 35°C. The residue (oleoresin) was dried and weighed; then dissolved in10 ml methanol for calculation of curcumin content using HPLC. In all extraction experiments acetone was used as the extraction solvent due to its high solubilization capacity. [10]

2.3. Preparation of Withania somnifera root extract

Isolation was carried out by using newer and conventional method. 200 gm ashwagandha punchang (root and leave) powder in to 2 litre round bottom flask (R.B.F.) then 750 ml petroleum ether was added to deffate the material. It was Refluxed at 60oC for 90 min. after 90 min materials was

filtrate and added further 800 mlof75 % Methanol and reflux continue for four hours at 60C. After that, flask was allowed to stand for cool and filtered all solutions. About 750 ml methanolic extract was collected, and transferred it into separation funnel. Now, extract with water (100ml) followed by dichloromethane (3×200ml) and collect the lower layer separate out in to 1 liter beaker (total 600ml solution). Now about 8 to 10 gms of activated charcoal were added into beaker and place beaker on water bath for 10 min. Then filter well by using whattmann filter paper. Yellowish color solution obtains after filtration, which allows evaporating on water bath in evaporating dish. Allowed to Complete dry it on water bath and then cool the evaporating dish for 10 min at room temperature.[11]

2.4. Preparation of Ocimum santum leaves extract

The maceration extraction method for ethanolic extraction involves ethanol (>99.5%). In a 1:10 ratio, the leaves powder was mixed with ethanol to make a 25% concentration (25g in 250ml of ethanol). The extract was filtered by Whatmanno.1filter paper after five days of soaking in solvent, then the extract subjected for further use.[12]

2.5. Preparation of polyherbal extract

The extract of Curcuma longa rhizome , Withania somnifera root and Ocimum santum leaves were taken in equal quantity (3 gm) and mixed together and polyherbal extract was obtained.

2.6. Physiochemical characterization and phytochemical study

Besides the authentication, the crude drugs were tested for the quality and purity parameters which include total Ash value, Acid insoluble value, Ash value, Water soluble ash, Sulphated ash, Loss on drying, Alcohol soluble extractive value, Water soluble extractive value, Petroleum ether extractive value, Foaming index. The herbal extracts so collected is evaluated for Carbohydrates, Proteins, Fats and Oils, Glycosides, Flavanoids, Alkaloids, Terpenoids, Steroids, Saponins, Tannins and Phenolic compounds.[13,14]

2.7. Thin layer chromatography

Thin layer chromatography was performed by the reported methods where various solvent solvent systems were tried and tested. Combination of Chloroform: Ethyl acetate: Ethanol (6:2:2) has identified as the suitable solvent system and the spots were detected using UV light at 254 nm.[15]

3. Pharmacological Study

3.1. Anti-inflammatory activity by bovine albumin fraction (In-vitro assessment)

3.1.1. Procedure

Reaction mixture 0.5 ml contains sample- A 0.05 ml in the concentration (500μ L/ml) or (1000 μL/ml) with 5% aqueous solution of bovine albumin fraction (0.450ml). The Ph (6.3) of solution was adjusted using small amount of 0.1 NH cl at 370C for 20min. then heat to 570C for 30 min. Cool the solution 0 measure the absorbance at 660 nm. Standard was used as Diclofenac sodium (1000μg/ml) and control contain 0.05 ml distilled water.

The percentage of inhibition of albumin denaturation was calculated by the following formula,

Percentage of inhibition (%) =[(A control – A sample) /A control] x 100

Where A control – Absorbance above all mixture except drug. A sample - Absorbance reaction mixture with Sample).[16]

3.2. Anti-inflammatory activity by Protein denaturation method (In-vitro assessment)

3.2.1. Procedure

The reaction mixture (10 mL) consisted of 0.4 mL of egg albumin (from fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4) and 4mL of Synthetic compound (1000 μg/ml). Similar volume of double-distilled water served as control. Then the mixtures were incubated at (370c ±2) in a incubator for 15 min and then heated at 70o c for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at concentration 1000 μg/ml was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula,

% Inhibition= Absorbance of control – Absorbance of test/Absorbance of control x 100[17]

3.3. Statistical analysis

All the data was expressed as Mean ± SEM. Statistical comparison was performed on graph pad prism, version 8.03 by one way ANOVA followed by Dunnett's multiple comparison test. Result *p ≤ 0.05. ** ≤ 0.001 were considered as statistically significant.[18]

4. Result and Discussion

4.1. Physiochemical characterization and Phytochemical study

The phytochemical analysis of the extracts has confirmed the presence of curcumin, withanolides, withanoferins and

linoleic acid responsible for majority of the Anti-diabetic potential.

4.2. Thin layer chromatography

The TLC has identified the flavonoids and curcumin, withanolides, withanoferins and linoleic acid in the polyherbal extract. The R_f value of polyherbal extract was found to be 0.82 which matches with the standard and hence presence of Charantin, Vicine and Polypeptide-p was confirmed.

4.3. Effect of polyherbal extract on Albumin denaturation

Table 1: Group wise % inhibition of albumin denaturation

Group	Conc. (μg/)ml	O. D	Mean	% Inhibition
Blank	-	0.25 0.29 0.30	0.28	-
(Standard) Diclofenac sodium	1000 μg/ml	0.13 0.12 0.15	0.13	95.35
(Test) Polyherbal extract	1000 μg/ml	0.12 0.13 0.10	0.11	60.71

Formula was used to determine the effect of polyherbal extract on inhibition of Albumin denaturation. Inhibition (%)= [(A control – A sample) /A control] x 100. Anti-inflammatory effect differs moderately between the polyherbal extract and the standard (Diclofenac sodium). A similar, more noticeable effect was observed when the Albumin denaturation was inhibited. Standard Diclofenac sodium inhibits the Albuminn denaturation by 95.35 % at a concentration of 1000 μg/ml. When compared to the Diclofenac sodium (standard), polyherbal extract was found to be efficient against Albumin denaturation, moderatley inhibiting at a concentration of 1000 μg/ml by 60.71 %. Thus, it has been determined that the polyherbal extract have the ability to inhibit the Albumin denaturation, which may be act as an Anti-inflammatory agent.

4.4. Effect of polyherbal extract on protein denaturation

The impact of the polyherbal extract on the suppression of Protein denaturation was calculated using a formula. % Inhibition= Absorbance of control – Absorbance of test/Absorbance of control x 100. There is little difference in the anti-inflammatory impact between the polyherbal extract and the reference (diclofenac sodium). At a dosage of 1000 μg/ml, standard Diclofenac sodium reduces Protein denaturation by 81.39%. The polyherbal extract was found to be less effective against Protein denaturation at a concentration of 1000 μg/ml, inhibited by 30.23% less than the normal dose of Diclofenac sodium. As a result, it has been established that the polyherbal extract can inhibit the Protein denaturation, which may have Anti-inflammatory properties.

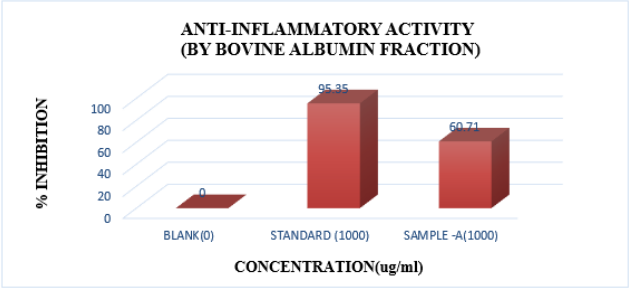

Figure 1: Effect of diclofenac sodium and polyherbal extract on Albumin denaturation

Table 2: Group wise % inhibition of Albumin denaturation

Group	Conc. (μg/)ml	O.D	Mean	% Inhibition
Blank	-	0.42 0.42 0.46	0.43	-
(Standard) Diclofenac sodium	1000 μg/ml	0.09 0.08 0.07	0.08	81.39
(Test) Polyherbal extract	1000 μg/ml	0.28 0.35 0.27	0.30	30.23

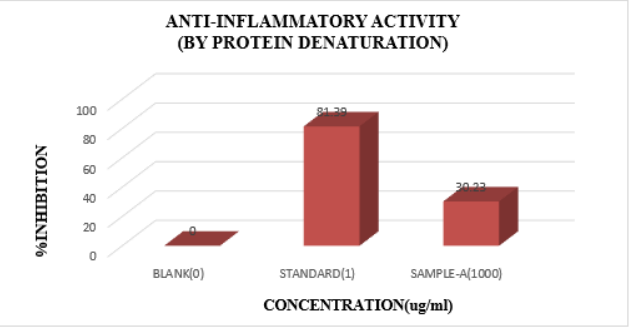

Figure 2: Effect of diclofenac sodium and polyherbal extract on protein denaturation

5. Conclusion

Denaturation of tissue protein is one of the well documented causes of inflammatory and arthritic diseases. Production of auto antigen in certain arthritic diseases may be due to denaturation of protein in-vivo. Agents that can prevent protein denaturation therefore couldbe worthwhile for anti-arthritic and anti-inflammatory drug

development. Polyherbal extract showed moderate activity when compared with standard drug. Furthermore, more research should be done on polyherbal extract to isolate and identify the phytochemical ingredients that reduce inflammation, as well as on experimental animals (In-vivo assessment) to corroborate the extract's anti-inflammatory properties.

6. Future Scope

1. In brief isolation and confirmation of phytochemical constituents should be done which are responsible for Anti-inflammatory effect.
2. Further animal study (In-vivo assessment) can be carried out on polyherbal extract of plants to confirmed Anti-inflammatory potential.
3. Exploring combination therapies with existing Anti-inflammatory medications to potentially enhance efficacy and mitigate side effects.
4. Tailoring treatment approaches based on individual patient profiles and genetic factors to maximize therapeutic outcomes.
5. Combining these Polyherbal extract may result in synergistic effects, enhancing their individual reduce inflammation and providing a more comprehensive approach to inflammation treatment.

7. Summary

The Anti-inflammatory potential study of polyherbal extract performed on Albumin and Protein denaturation and standard procedure given in references. The results of critical in-vitro assessments have convinced the moderate efficacy of the polyherbal extract against inflammation.

8. Conflict of Interest

The authors declare that there is no conflict of interest.

9. Source of Funding

None.

10. Conflict of Interest

None.

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References

1. Kadhim H, Tabarki B, Verellen G, De Prez C, Rona AM, Sébire G. Inflammatory cytokines in the pathogenesis of periventricular leukomalacia. *Neurology*. 2001;56(10):1278–84.
2. Sabio G, Davis RJ. MAP kinase signaling pathways. *Seminin Immunol*. 2014;26(3):237–45.
3. Huang. HMGB1, a potent proinflammatory cytokine in sepsis. *Cytokine*. 2010;51(2):119–26.
4. Ferrero-Miliani. Chronic inflammation: importance of NOD 2 and NALP 3 in interleukin-1β generation. *Clin Exp Immunol*. 2007;147:227–235.
5. Nathan C. Non resolving inflammation". *Cell*. 2010;140:871–882.
6. Anbalagan K, Sadique J. Influence of an Indian medicine (Ashwagandha) on acute- phase reactants in inflammation. *Indian J Exp Biol*. 1981;19(3):245–9.
7. Anbalagan K. Role of prostaglandins in acute phase proteins in inflammation". *BiochemMed*. 1984;31(2):236–45.
8. Singh N, Nath R, Lata A, Singh SP. Withania somnifera (ashwagandha), a rejuvenating herbal drug which enhances survival during stress", (anadaptogen). *Int J Crude Drug Res*. 1982;20(1):29–35.
9. Ammon H. Mechanism of anti-inflammatory actions of curcumin and boswellic acids. *J Ethnopharmacol*. 1993;38(2-3):113–9.
10. Apisariyakul A, Vanittanakom N. Anti-fungal activity of turmeric oil extracted from Curcuma longa (Zingiberaceae). *J Ethnopharmacol*. 1995;49(3):163–9.
11. Decastro M. Soxhlet extraction of solid materials: an out dated technique with a promising innovative future. *Anal Chimica Acta*. 1998;369(1-2):1–10.
12. Rajesh H, Rao SN, Shetty P, Ran M, Rejeesh E, Joseph L, et al. Phytochemical analysis of aqueous extract of Ocimum sanctum linn. *Int J Univ Pharm Sci*. 2013;2(2):462–8.
13. Kokate CK. Textbook of Pharmacognosy. vol. 14. and others, editor. Nirali Publication; 2017. p. 145.
14. Dr KR, Khandelwal. Practical Book of Pharmacognosy &. *Phytochemistry"*. 2016;18:3–18.
15. Wagner H, Bladt S. Plant drug analysis: a thin layer chromatography atlas. vol. 2. Berlin: Springer; 1996. p. 20–40.
16. Ameena M, Arumugham M, Ramalingam K, Rajeshkumar S. Evaluation of the Anti-inflammatory, Antimicrobial, Antioxidant, and Cytotoxic Effects of Chitosan Thiocolchicoside-Lauric Acid Nanogel", . *Cureus*. 2023;15(9):46003.
17. Fasse S, Gottschalk A. In Vitro Analysis of the Anti-Inflammatory Activity in Dairy Products Containing Salicornia Europaea". *Chem Eng Transac*. 2023;102:73–8.
18. Quirk T, Quirk MH, Hortan HF. One-Way Analysis of Variance (ANOVA). In: Excel 2016 for Biological and Life Sciences Statistics. New York, NY: Springer; 2012. p. 163–79.

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