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Phyatochemical and Pharmacological Evaluation of Some Indigenous Plants for Rheumatoid Arthritis

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Abstract: *Rheumatoid arthritis is a joint inflammation characterized by increased cellularity of synovial tissue. It is distinguished by pain, swelling, and stiffness of the synovial joints. Approximately 1% of worldwide population is affected by Rheumatoid Arthritis (RA). Females have been three instances greater affected than men. It is usually occurring in the age of 35-60 years. Arthritis is a common prolonged health issue that leads to morbidity. Symptoms include discomfort, soreness, reduced capacity of joint movement, warmth and skin redness, dry eyes, mouth, and firm lumps under the skin in places like the elbows and arms known as rheumatoid nodules. Arthritis is of particularly five kinds – Osteo arthritis, Rheumatoid Arthritis, Psoriatic Arthritis, Gout and Lupus.*

Allopathic drugs such as NSAIDs, Celecoxib, and Duloxetine has been used with adverse consequences. Due to increased safety and efficacy, the use of herbal medicines to combat these side effects has increased. Synthetic formulations could only provide symptomatic relief; they could not cure Rheumatoid arthritis, which is a critical need. The proposed formulation is a good substitute for synthetic topical formulations like Diclofenac sodium and can be combined with other in vivo formulations to completely cure Rheumatoid arthritis. Emulgel is one of the formulations that can help in achieving this goal. The current study aimed to formulate a polyherbal emulgel and to assess its antioxidant, anti-inflammatory, and antiarthritic activity in vitro and in vivo respectively..

Keywords: *Rheumatoid arthritis*.

INTRODUCTION

HISTORY OF MEDICINAL PLANTS

Plants were cultivated as medicinal plants approximately 60,000 years in the past. In India, China and Egypt approximately 5000 years in the past and in Greece and central Asia about 2,500 years ago. Egyptians and Chinese have been using plants for therapeutic purposes from over 27 centuries BC Anget al. [15]. Ancient Greeks were also acquainted with medicinal plants uses. Then Hippocrates and Aristotle used those medicinal plants for remedy of sicknesses. In 75-74 BC, physician Pedanius Dioscorides wrote “De Materia Medica”, an encyclopedia that explained six hundred remedial traditional medicines Lindberg and Berteslen [173], Rios and Recio [257], A Zargari [332]. Fifteenth and sixteenth century was a flourishing era for natural medication. The early nineteenth century was a turning point in natural drug treatments with isolation of Alkaloids and Glycosides. The therapeutic utilization of natural drugs declined steadily in the nineteenth and twentieth centuries Sewell and Kopaei [273].

CONVENTIONAL MEDICINAL SYSTEM

“World Health Organization” (WHO) described indigenous herbal plants were derived from natural sources that can be utilized without commercial processing for the remedy of illnesses locally as summarized in Fig. 1.1 Tibort and Kaptchuk [305]. Conventional herbal remedies were utilized in emerging and industrialized nations due to less complications M Wichtl [322]. “Complementary or alternative medicine” attribute to the growing use of conventional

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medicines in affluent countries. “Traditional Chinese Medicine” (TCM) is currently the most prominent among all conventional medical practices worldwide, accompanied by Medicine in India. In developed countries, Oriental medicine belongs to Chinese, Japanese and Korean drug treatments decided on by nationals from Korea, while Asian medicine mostly consists of Traditional ayurvedic and Tibetian medication WJHLiu [174].

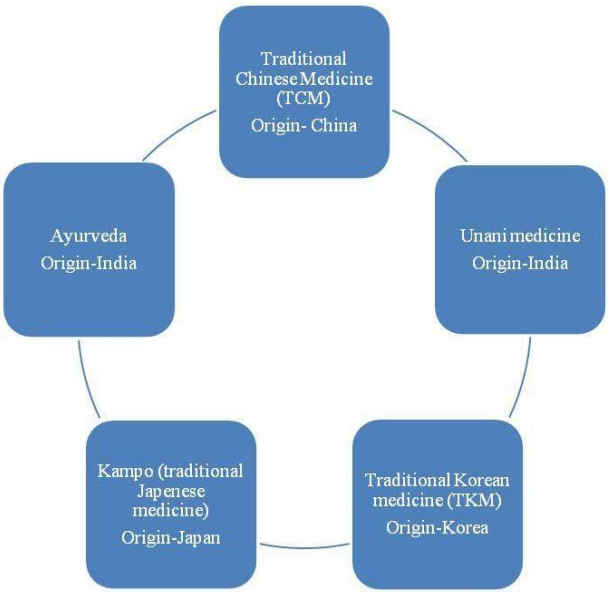

Fig. 1.1 Traditional Medicinal Systems

ROLE OF NATURAL PRODUCTS

Nature is constantly a golden sign to expose the distinguished phenomena of coexistence. Existence will always be a perfect example of the distinct phenomena of mutual cooperation. The foundation for curing illnesses in humans was based on herbal products derived from floral, animals, and mineral resources Firenzouli and Gouri [91]. Medicinal flora has certainly been taken into consideration with the aid of human beings due to the fact historic instances. Medicinal flora was utilized by international countries like China, Greece, Egypt and India AC Hamilton [117]. More than a tenth of species of plants are used in preparation of medicinal and cosmetic formulations. The term "Medicinal plant" describes a variety of plants which possess therapeutic potential. Distinct types of seeds, roots, leaves, fruits, pores and skin, plants, or entire plants could be used. The toxic effects and unfavorable effects of traditional and allopathic medications were also key attributes in the unexpected increase in population requirements and growth within the wide range of natural drug producers BA Hassan Rasool [249].

II. PLANT PROFILE & REVIEW OF LITERATURE

1. PLANT PROFILE OF NARDOSTACHYS JATAMANSI DC.

Botanical Description Kingdom - Plantae
Division - Mangnoliophyta
Class - Mangnoliopsida
Order - Dipsacales
Family - Valerianaceae
Genus - Nardostachys
Species - N. jatamansi

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Biological Source
It is obtained from dried roots and rhizomes of Nardostachys jatamansi DC.
Family: Valerianaceae

Fig. 2.1 Nardostachys jatamansi rhizome

Vernacular Names Satyavati
English-Indian Spikenard
Hindi-Jatamansi
Bengali-Jatamansi
Tamil-Jatamashi
Punjabi-Belcher
Marathi-Jatamavshi
Telugu-Jatamanshi

Fig. 2.2 Andrographis paniculata leaves

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Vernacular NamesBasu
English-Creat
Hindi-kalmegh
Bengali-Kalmegh
Tamil-Gopuram thangi
Marathi-Banchimani
Telugu-Uanadi

PLANT PROFILE OF CELASTRUS PANICULATUS
Botanical Description
Kingdom - Plantae
Division - Tracheophytes
Class - Rosids
Order - Celaestrales
Family - Celastraceae
Genus - Celaestrus

Biological Source
It consists of dried seeds of Celaestrus paniculatus, Family: Celastraceae

Fig. 2.3 Celastrus paniculatus plant along with seeds

III. PLAN OF RESEARCH WORK

1. Collection, Authentication, and Processing of Plant Materials
Collection of Nardostachys jatamansi (rhizomes), Andrographis paniculata (aerial parts), and Celastrus paniculatus (seeds) from authenticated sources followed by botanical authentication, cleaning, drying, pulverization, and storage under suitable conditions.

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2. Extraction of Phytoconstituents

Preparation of extracts using suitable extraction techniques such as maceration, Soxhlet extraction, or solvent extraction employing different solvents based on polarity to obtain maximum yield of bioactive constituents.

3. Phytochemical Investigation

Qualitative phytochemical screening for identification of major secondary metabolites including alkaloids, flavonoids, glycosides, tannins, phenolics, saponins, steroids, and terpenoids.Quantitative estimation of important phytoconstituents. Isolation and purification of active compounds using chromatographic techniques. Characterization and identification of isolated compounds using analytical techniques such as TLC, HPLC, FT-IR, UV spectroscopy, and other suitable methods.

4. Formulation Development

Development of a suitable polyherbal topical formulation such as emulgel/gel/ointment incorporating optimized herbal extracts for enhanced therapeutic effectiveness, patient acceptability, and stability.

5. Evaluation and Optimization of Formulation

Evaluation of the developed formulation for various physicochemical parameters including:

- Appearance and homogeneity
- pH determination
- Viscosity
- Spreadability
- Drug content uniformity
- Extrudability
- Stability studies under different storage conditions according to standard guidelines.

IV. AIMS AND RESEARCH WORK

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder characterized by progressive destruction of synovial joints, severe pain, swelling, stiffness, and reduction in mobility. Continuous inflammation results in cartilage degradation, bone erosion, and long-term disability, thereby affecting the quality of life of patients. Although several synthetic anti-arthritic drugs such as NSAIDs, corticosteroids, and DMARDs are available for treatment, their prolonged use is associated with serious adverse effects including gastric irritation, hepatotoxicity, nephrotoxicity, hypertension, immunosuppression, and cardiovascular complications. Therefore, there is a growing demand for safer and more effective herbal alternatives with minimum side effects.

Medicinal plants have gained considerable attention due to the presence of bioactive phytoconstituents such as flavonoids, alkaloids, terpenoids, phenolics, glycosides, tannins, and steroids possessing anti-inflammatory, antioxidant, immunomodulatory, and antiarthritic properties. In the present research work, an attempt is made to develop a polyherbal formulation containing Nardostachys jatamansi, Andrographis paniculata, and Celastrus paniculatus for the management of rheumatoid arthritis.

The major aim of the study is to investigate the synergistic antiarthritic potential of selected medicinal plants and to formulate a stable, safe, and effective herbal topical preparation capable of reducing inflammation, oxidative stress, pain, and joint degeneration associated with rheumatoid arthritis. The study also focuses on isolation and characterization of active phytoconstituents responsible for therapeutic activity.

Furthermore, the research intends to evaluate the formulation through phytochemical analysis, in vitro antioxidant and anti-inflammatory studies, and in vivo antiarthritic screening using suitable experimental models. The developed herbal formulation may serve as a potential alternative to conventional synthetic topical preparations such as Diclofenac sodium with improved safety, enhanced patient compliance, and reduced toxic effects during long-term therapy.

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The present work also aims to contribute toward scientific validation of traditional medicinal plants and to encourage the utilization of herbal therapeutics in the treatment of inflammatory joint disorders.

V. PHYTOCHEMICAL EVALUATION

1. MATERIALS AND METHODS

1. Plant Material

The roots and rhizomes of Nardostachys jatamansi (N. jatamansi) were procured from the local herbal market of Haridwar, Uttarakhand, India during October 2014. The aerial parts of Andrographis paniculata (A. paniculata) and seeds of Celastrus paniculatus (C. paniculatus) were procured from Yucca Enterprises, Mumbai, Maharashtra, India during February 2015.

All plant materials were thoroughly cleaned to remove foreign matter, dust, and other impurities. The collected plant materials were shade dried at room temperature to prevent decomposition of thermolabile constituents and preserve phytochemical integrity. The dried materials were then coarsely powdered separately using a mechanical grinder and stored in airtight containers protected from moisture, light, and contamination until further use.

2. Identification and Authentication

The collected plant materials were identified and authenticated by Dr. (Mrs.) Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum Delhi (RHMD), CSIR-NISCAIR, New Delhi, India. Voucher specimens of all plant materials were deposited in the herbarium for future reference and authentication purposes.

The authentication ensured the botanical identity, purity, and quality of the selected medicinal plants used in the present investigation.

3. Preparation of Extracts

The powdered plant materials were subjected to Soxhlet extraction using ethanol as a solvent because of its excellent ability to extract both polar and moderately non-polar phytoconstituents.

Approximately 1 kg of powdered rhizomes of N. jatamansi was extracted continuously with ethanol in a Soxhlet apparatus until complete exhaustion of the marc. Similarly, 1 kg of powdered aerial parts of A. paniculata and 1.5 kg of powdered seeds of C. paniculatus were extracted separately using ethanol under identical conditions.

The obtained extracts were filtered and concentrated under reduced pressure using a rotary vacuum evaporator to remove excess solvent. The concentrated extracts were further dried to obtain semisolid masses and percentage yields were calculated with respect to the air-dried plant material. The dried extracts were stored in airtight amber-colored containers at refrigerated temperature until further phytochemical and pharmacological investigations.

QUALITATIVE PHYTOCHEMICAL SCREENING

The extracts of N. jatamansi, A. paniculata and C. Paniculatus were analyzed for the presence of various phytoconstituents such as alkaloids, carbohydrates, glycosides, flavonoids, proteins, steroids, and saponins Trease and Evans [307].

TEST FOR ALKALOIDS

After the extract had been evaporated, the residue was shaken with diluted HCl. The filtrate was subjected to the following tests:

• Dragendorff's Test

Potassium bismuth iodide (Dragendorff's reagent) was added to the filtrate, and the formation of reddish-brown ppt. indicates the presence of alkaloid.

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• Mayer's Test
Potassium mercuric iodide (Mayer's reagent) was added to the filtrate, and the formation of cream ppt. shows the presence of alkaloid.

• Wagner's Test
Iodine potassium iodide (Wagner's reagent) was added to the filtrate, and the development of a reddish-brown ppt. reveals the presence of alkaloid.

• Hager's Test
Iodine picric acid solution (Hager's reagent) was added to the filtrate, and the emergence of reddish yellow ppt. confirms the presence of alkaloid.

CARBOHYDRATES
• Molisch's Test
2-3 drops of α-naphthol were added to the test solution. Then few drops of Conc. Sulphuric acid was added from the sides of test tube, formation of violet color ring at the junction indicates presence of Carbohydrates.

• Fehling's Test
To the test solution, Fehling solution A and B was mixed in equal amount and was boiled for 10 minutes, formation of yellow and then brick red ppt. indicates the presence of reducing sugars.

• Benedict's Test
To the test solution, Benedict's solution was added in equal quantity and was mixed by heating for 10 minutes, formation of yellow color indicates the presence of sugar.

FLAVONOIDS
• Shinoda Test
To the filtrate, 5 ml of ethanol was added. Few drops of Conc. HCl and 0.5 g of magnesium metal were added in it, formation of pink color indicates the presence of flavonoids.

• Zinc Chloride Test
To the extract, zinc metal was added, few drops of Conc. HCl was added in it, formation of brick red color indicates the presence of flavonoids.

TANNINS
• Ferric Chloride reagent
To the test solution, FeCl3 solution was added, formation of deep blue or dark green ppt indicates the presence of tannins.

• Lead acetate Test
To the test solution, 10% w/v of lead acetate in distilled water was added, ppt. was obtained which indicates the presence of tannins.

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PROTEINS

• Biuret's Test

Test solution was treated with equal volume of 1% sodium hydroxide and few drops of aqueous copper sulfate (Biuret's reagent), formation of purple color indicates the presence of proteins.

STEROIDS

• Salkowski's Test

2 ml of filtrate was treated with 2 ml of chloroform and 2 ml of sulphuric acid, formation of red chloroform layer and yellow acidic layer indicates the presence of sterols.

• Legal Test

The concentrated ethanolic extract was made alkaline with few drops of 10% sodium hydroxide and then freshly prepared sodium nitroprusside solution was added, formation of blue color indicates the presence of glycosides.

SAPONINS

• Foam test

Few mg of test residue was shaken with a small amount of sodium bicarbonate and water in a test tube, formation of foam indicates the presence of saponins.

VI. PHARMACOLOGICAL SCREENING

ARTHRITIS

Arthritis refers to the inflammation of one or more joints, causing pain and stiffness. This is also known as Rheumatic disorder and the science which deals with these types of conditions is called Rheumatology.

Types of arthritis

There were several types of Arthritis which was summarized in Table

Table :- Classification of Arthritis with causes, symptoms and diagnosis

S.No.	Type	Causes	Symptoms	Diagnosis
1.	Osteoarthritis	Cartilage damage	Pain, swelling, warmth and stiffness	X-rays, MRI and Blood-tests

2. Maturation Stage

At the region of secondary lymphoid tissues or bone marrow, this stage begins. This involves the elicitation of immunological responses against endogenous epitopes, which leads to the production of self-antigens. This will induce triggering of "MHC class II-dependent T cells" which will excite "B cells" to produce more ACPA, eventually cause pain and inflammation A Krishnamurthy [156], G Wigerblad [323].

3. Targeting Stage

In this stage synovitis occurs with joint swelling and synovial membrane inflammation. The synovial compartment is infiltrated by leucocytes and inundation of inflammatory mediators in the synovial fluid. The involvement of "fibroblast-like synoviocytes" (FLSs) with cells such as monocytes, macrophages, mast cells, DCs, T-lymphocytes, and B cells ultimately results in a cascade of inflammation Burmester et al. [52].

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a) Monocytes/ macrophages – Causes infiltration of synovial membrane, production of TNF-α and NF-kB mediated by ACPA and α-enolase induced production of inflammatory mediators

b) Mast cells- Due to activation by ACPA and TRLs ligands results into production of inflammatory mediators cytokine interleukin (IL)-17A A.J Hueber [125]

c) T-cells- Proliferation of T-cells, due to accumulation of DCs ACPA Zvaifler

VII. CONCLUSION

The various qualitative phytochemical tests indicated the presence of Alkaloids, Carbohydrates, Flavonoids, Proteins and Saponins in N. jatamansi, Alkaloids, Flavonoids, Tannins, steroids and Saponins in A. paniculata and Alkaloids, Flavonoids, Tannins and Steroids in C. paniculatus. The various phytoconstituents were also observed in different extracts of Nardostachys jatamansi, Andrographis paniculata and Celaestrus paniculatus by using HPTLC which further requires isolation and characterization.

Many volatile oils have recently been discovered to be beneficial in the management of various illnesses. The GC and GC-MS analytical techniques were very useful technique in qualitative and quantitative estimation of volatile constituents. Seven Sesquiterpenes were found, out of which five were hydrocarbons i.e. calarene, vardiflorene, α-panasinsen, α- santalene, γ- himachelene, one is ketonic i.e. jatamansoneand one is alcoholic i.e. epiglobulol ; one Ttriterpene was observed i.e. unknown ; among two others Ionol 4 (9.9%), 2,2,7,7- Tetramethyl tricyclo[6,2,1,0 (1,6)]undec-4-ene 3-one (1.7%) was observed.

Two labdane diterpenoids i.e, andrographolide, neoandrographolide and one flavone i.e, 5-Hydroxy 7-methoxy flavone were isolated successfully from the Ethanolic extract of Andrographis paniculata. Furthermore, these isolated compounds needs to be evaluated for antioxidant and anti-inflammatory activity.

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