PHYTOCHEMICAL, ANTI-INFLAMMATORY AND WOUND HEALING ACTIVITY OF HOMEOEOPATHIC DRUG *ARNICA MONTANA Ø* AND ITS CRUDE EXTRACT – A COMPARATIVE STUDY

INTRODUCTION:

The first remedy that strikes the mind of a Homoeopathic Physician in management of any types of injury, falls, blows, concussions, contusions, fracture, sprain, strain, trauma etc., is the Homoeopathic Drug '*Arnica Montana'.* This Homoeopathic drug has inherent capacity to cure acute and long standing effects of even remote injuries or trauma hence, considered as the homeopathic anti-trauma remedy par-excellence.[1,2,3]

The whole plant especially flower heads of Arnica Montana have been used traditionally over years for treatment of inflammatory conditions administered either orally or topically.[4,5] Arnica is also used post operatively in cosmetic and dental surgeries. Arnica Montana is reported to have anti-inflammatory, analgesic, hemostatic, wound healing and anti-septic properties. Hence used in treatment of Osteoarthritis, bruises, contusions, edema, hematoma, insect bites, joint pain (including rheumatic conditions), sprains, muscle soreness. [6,7]

The therapeutic properties of various plants are mainly due to their Phytoconstituents. Development of science and technology has brought to light many mysterious entities and mechanisms of nature in recent years. Similarly, the identification and separation of various secondary metabolites, the *Phytochemical components* from the plants and employing them for therapeutic purposes is one of the major milestone in development of Pharmacognosy.

Numerous active compounds are identified from various parts of the Arnica plant like tannins, essential oil, fatty acids, thymol, pseudoguaianolide, flavonoids, carotenoids, polysaccharides, phenolic acids, terpenoids, caffeic acid derivatives and sesquirterpenic lactones[6,8,10].There are studies presenting the chemical diversity of these phytochemicals in samples obtained from various habitat.[9] This phytochemical study on Homoeopathic Mother tincture and its crude extract will open a new path to qualitative comparative study on various Homoeopathic drugs.

NEED FOR STUDY:

The Phytochemicals play a pivotal role in growth, development and protection of plants, hence called secondary metabolites. Phytochemical screening is essential to identify the therapeutically important components available in the particular plant. Though there are phytochemical studies on Arnica, that shows the anti-inflammatory action is mainly due to the presence of Sesquiterpene lactones in the flower heads and high amount of caffeic acid derivatives in whole plant[6,10] whereas the wound healing property is mainly due to the presence of Helenalin, a Sesquiterpene lactones[11]. The synergistic action of all the phytochemicals in the plant is considered superior to the medicinal property exerted by single isolated constituent.[14]

Few studies have been conducted to show diversity of phytoconstituents of different parts of particular plant as well as that of different locations. In 2019, Kimel et al., for the first time performed a comparative study on quantitative chemical analysis of Tincture of Arnica whole plant and its flowers. This study revealed presence of caffeic acid derivatives, Sesquiterpene lactones and flavonoids with slight variations in quantity.[10,11]

Most of the studies in-vitro, in-vivo as well as clinical studies were carried out using various potencies to experiment the anti-inflammatory, wound healing, analgesic action and alleviation of post-operative ailments[12,13]. But there are no studies reported comparing the phytochemicals in crude extract and mother tincture highlighting their anti-inflammatory and wound healing properties. So, this study is taken up to fulfill this gap in Homoeopathic phytochemical research.

AIMS & OBJECTIVES:

1. To explore the *in vitro* wound healing and anti-inflammatory action of *Arnica Montana* Ø and its crude extract.
2. To compare secondary metabolites, the Phytochemicals available in the *Arnica Montana* Ø and its crude extract.

METHODOLOGY:

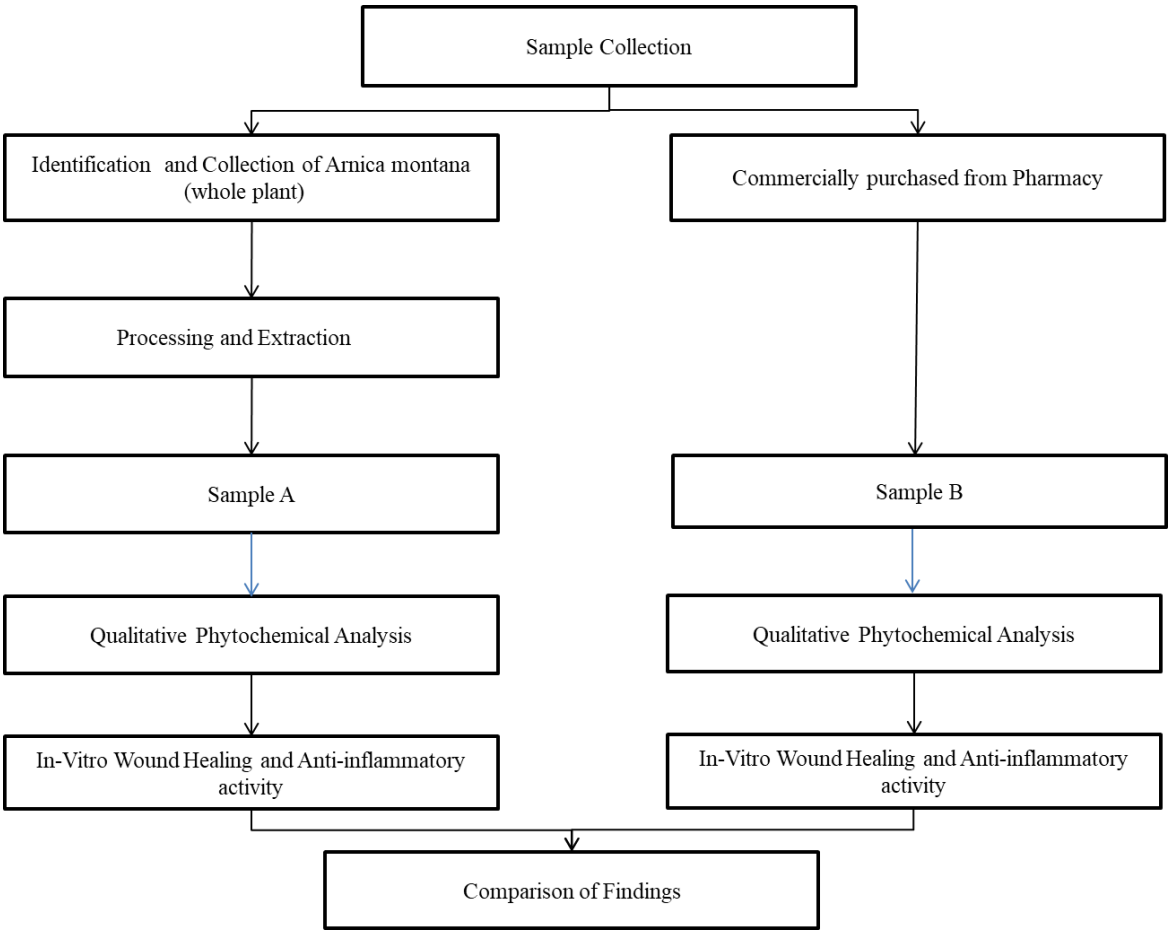

Fig: Overall Methodology

i) SAMPLE COLLECTION AND PROCESSING

SAMPLE A:

Sample identification:

The Arnica montana plant will be identified by an experienced Botanist for collection and preparation of crude extract.

Sample collection:

The whole plant of Arnica Montana plant will be collected from the natural habitat and dried in shade to remove the moisture.

Sample Preparation:

The dried whole plant will be powdered and treated with ethanol in a conical flask and left undisturbed for 48 hrs. This mixture will be then subjected to filtration.

The solid remnant will be retained in a linen bag inside the thimble of the Soxhlet apparatus and the filtrate is placed in boiling flask. The solvent / filtrate in boiling flask will be heated until a colorless solvent is obtained in the extractor.

The process will be repeated by removing the solid remnant for deriving the crude extract of Arnica Montana (Sample A). This crude extract will be cooled and transferred to a Screw cap tube (glass).

SAMPLE B:

The sample B will be commercially purchased from **Willmar Schwabe India Pvt Ltd**., hence sample identification, collection and preparation are required.

ii) PHYTOCHEMICAL ANALYSIS OF SAMPLE A & B:

Test for Carbohydrates, reducing Sugars, Amino Acids, Proteins, Vitamin C, Tannins, Alkaloids, Flavonoids, Phlobatannins, Steroids, Terpenoids, Phenolic Compounds, Saponins, Glycosides and Anthroquinones will be performed for both Sample A & B. The secondary metabolites available in Sample A & B will then be subjected to Qualitative Comparative study.

iii) IN-VITRO ACTIVITY OF WOUND HEALING AND ANTI-INFLAMMATORY ACTION OF SAMPLE A & B:

a) Anti-inflammatory action of Sample A & B:
   The anti-inflammatory action of Sample A & B will be studied by Inhibition of Albumin denaturation and Proteinase inhibitory assay.

b) Wound Healing action of Sample A & B:
   The wound healing action of Sample A & B will be studied by
   1) HaCaT Keratinocytes
   2) Human dermal fibroblasts.

The findings of in-vitro study of anti-inflammatory and wound healing activity of Sample A & B will then be subjected to comparison.

IMPLICATIONS:

This study will develop knowledge and insight among Pharmacognosists as well as Homoeopaths

- Whether there is significant difference in anti-inflammatory and wound healing activity of the crude extract and Arnica Montana Ø.
- Whether the Phytochemicals present in Homoeopathic Mother tincture is similar to that of the crude extract.
- to explore the synergestic action by the phytochemicals retained in the Homoeopathic mother tincture against that of an isolated component and open a new path to conduct researches on this area.

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