Abstract:
Natural medicines were the useful option for the prevention of various human diseases. The price of natural products is low compared to synthetic products. Feverfew (Tanacetum parthenium) is important medicinal plants with different pharmacologic activities. Parthenolide (PN) is main active constituent in feverfew leaves which belongs to sesquiterpene lactone compounds. The leaves have been used to treat a wide range of diseases such as menstrual disorders, asthma, migraine, fever, arthritis, insect bites. Therefore, it is very crucial to develop effective and selective methods for the extraction of the feverfew leaves. This paper intended to provide a comparison of the variety of methods used in extraction using different solvents (water, ethanol, methanol). The Anti-inflammatory activity of feverfew leaves was measured by inhibition of hypotonicity induced albino rat red blood cells membrane lysis (Heat Induced Hemolysis) in the extract. Result showed that, In the proposed method of extraction, ultrasound assisted extraction gives efficient amount of yield as compared to other methods. The potency of the ethanolic leaves extract was compared with standard diclofenac sodium.

Keywords: Feverfew, Tanacetum parthenium, Parthenolide, Heat induced hemolysis, In-vitro anti-inflammatory activity.

1. INTRODUCTION

The word, “FEVERFEW” derives from the Latin word febrifuge, meaning, “FEVER REDUCER”. Tanacetum parthenium, is flowering plant in the daisy family, Asteraceae. It is a traditional medical herb that is used commonly to treat fever and inflammation [1]. This plant is native to Kazakhstan, Central Asia and Mediterranean region, but it is found in different parts of Europe, Asia, America [2,3]. Sesquiterpene lactones is the major secondary metabolites has been reported in the feverfew plant, of which Parthenolide is main active chemical constituent majorly, presents in leaves (0.95%) [2,5]. It is wellknown to have anti-inflammatory property [4]. Several methods have been reported to extract the bioactive compounds in feverfew leaves, in which Ultrasound assisted extraction, Soxhlet extraction, Maceration, Bottle stirring method has been used for the extraction of Parthenolide. The extraction methods have been carried out with the objective to obtain the maximum concentration of active chemical constituents, in short period of time using different solvents (water, ethanol, methanol) [6,7]. Inflammation is the reaction to the injury of cells and tissues through various factors like infection, chemicals, heat and mechanical injuries. The anti-inflammatory drugs are inhibitors of cyclo-oxygenase pathway of arachidonic acid metabolism which generates prostaglandins which are said to be the mediators of inflammation.

2. MATERIAL AND METHODS

2.1 Plant material
Inhibition of prostaglandins synthesis is essential to treat inflammation [8]. Ethanolic extract of Parthenolide helps to prevent excessive clumping of platelets and inhibits the release of certain chemicals, including serotonin, prostaglandin and some inflammatory mediators [9,10]. Leaves of the plant, Tanacetum parthenium L., were collected from INDIAMART. The taxonomic identity of the plant was confirmed by Botanical Survey of India. Leaves were dried for 72 h. Dried leaves were powdered (180-355 μm) using a lab mill and stored in polyethylene bag at room temperature.

2.2 Extraction
It is separation of medicinally active portions of plant using selective solvents through standard procedures. The purpose of all extractions is to separate the soluble plant metabolites, leaving behind the insoluble cellular (residue). The selection of solvent is one of the important steps for extraction. Selectivity, solubility, cost and safety should be considered in the selection of solvents [11].

A) Maceration: This is a very simple extraction method with the disadvantage of long extraction time and low extraction efficiency. The powdered crude material is placed in a stoppered container with the solvent (water, ethanol, methanol) and allowed to stand at room temperature for 24-48 hours with frequent agitation until soluble matter is dissolved. Mixture is then strained the Marc pressed and may be macerated again. Combined liquid is clarified by filtration or decantation after standing [12].

B) Soxhlet extraction: This is an automatic continuous, more efficient and required less extraction time and solvent as compare with maceration. 20 gm of crude powered leaves was placed in a muslin cloth bag, which is placed in thimble of Soxhlet apparatus. The extraction solvent (ethanol, methanol, water) in flask was heated (<80–100°C) and the vapours condensed in condenser. The condensed extract dips into the thimble containing the crude drug. Continue the process until the colour of solvent was not change or not to become colourless. Filter the solvent. Evaporate filtrate to get extract [11,13].

C) Ultrasound assisted extraction: Ultrasound in the solvent producing cavitation accelerates the dissolution and diffusion of the solute as well as the heat transfer, which improves the extraction efficiency of product. It includes low solvent and the reduction of extraction temperature and time [14].
Ultrasound, with levels greater than 20 kHz, is used to disrupt plant cell walls, which helps to improve the solvent’s ability to penetrate the cells and obtain a higher extraction yield. Take 10 gm dried and powdered leaves. Sonicated with ethanol, methanol, water for 30,60, 90, 120 minutes. Filter the solvent. Evaporate filtrate to get extract [11, 14].

**D) Bottle stirring method:** 20 gm of powdered drug was placed in beaker containing 200 ml of solvent and beaker was covered with the aluminum foil. Stirring done with 7 h with constant speed. Filter the solvent. Evaporate filtrate to get extract [6, 15, 16]. After extraction, the extracts were tested to find out presence of various bio-active compounds using standard (std) phytochemical method of testing [17, 18].

### 2.3 ANTI-INFLAMMATORY STUDY

#### HEAT-INDUCED HEMOLYSIS STUDY: (Membrane stabilization method)

1. Preparation of anticoagulants: - 1.15 mg of disodium salt of EDTA add in 10 ml of water. Use 0.1 ml per 1 ml of blood.
2. RBC suspension: - Collect 1 ml blood from albino rat + 9 ml EDTA add in 10 ml of water. Use 0.1 ml per 1 ml of blood.

#### 2.3.1 Extraction:

**SOXHLET EXTRATION METHOD:**
1. ETHANOL
2. METHANOL
3. WATER

**BOTTLE STIRRING METHOD:**
1. ETHANOL
2. METHANOL
3. WATER

**MACERATION:**
1. ETHANOL
2. METHANOL
3. WATER

**ULTRASONICATION**
1. ETHANOL
2. METHANOL
3. WATER

Among different solvents and different method of extraction used, Ultrasound assisted extraction with ethanol solvent was found a maximum % yield in minimum time [table no. 1].

#### 3.2 Phytochemical screening

In preliminary phytochemical screening, the ethanol extract of feverfew leaves demonstrated the presence of alkaloids, flavonoids (18.92%), tannins (22.18%), saponins and glycol sides.

#### 3.3 Invitro anti-inflammatory study

The in-vitro anti-inflammatory activity of ethanolic extract of feverfew leaves are presented in Table no 2. It was observed that the increase in the concentration of leaves extract increases the activity of membrane stabilization. The inhibitory activity of ethanolic extract of feverfew leaves showed that 83.08% inhibition at a concentration 5 mg/ml, which was fine comparable to standard Diclofenac sodium.

<table>
<thead>
<tr>
<th>SR NO</th>
<th>CATEGORY</th>
<th>% INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>STD. (1 mg/ml)</td>
<td>66.48 %</td>
</tr>
<tr>
<td>3</td>
<td>Test sample I (1 mg/ml)</td>
<td>64.95 %</td>
</tr>
<tr>
<td>4</td>
<td>Test sample II (3 mg/ml)</td>
<td>74.10 %</td>
</tr>
<tr>
<td>5</td>
<td>Test sample III (5 mg/ml)</td>
<td>83.08 %</td>
</tr>
</tbody>
</table>

#### 3. RESULTS AND DISCUSSION

#### 3.1 Extraction: Extraction was carried out triplicate time by using drug: solvent (1:10) ratio. Comparison between average % yield of all extracts obtained by different methods of extraction is given in Table no. 1

#### Table 1. Average % yield of feverfew leaf extracts

<table>
<thead>
<tr>
<th>SR NO</th>
<th>EXTRACTION METHOD</th>
<th>% YIELD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SOXHLET EXTRACTION METHOD: 1) ETHANOL 2) METHANOL 3) WATER</td>
<td>1) 18.90% 2) 13.8% 3) 15.13%</td>
</tr>
<tr>
<td>2</td>
<td>BOTTLE STIRRING METHOD: 1) ETHANOL 2) METHANOL 3) WATER</td>
<td>1) 20.90% 2) 18.8% 3) 17.33%</td>
</tr>
<tr>
<td>3</td>
<td>MACERATION: 1) ETHANOL 2) METHANOL 3) WATER</td>
<td>1) 11.5% 2) 9.05% 3) 10.89%</td>
</tr>
<tr>
<td>4</td>
<td>ULTRASONICATION 1) ETHANOL 2) METHANOL 3) WATER</td>
<td>1) 22.18% 2) 18.67% 3) 14.52%</td>
</tr>
</tbody>
</table>

#### 4. CONCLUSION

In the proposed method of extraction, Ultrasound assisted extraction of feverfew plant poses significant anti-inflammatory activity when compared with standard drug.

#### 5. ACKNOWLEDGEMENTS

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#### 6. REFERENCES


