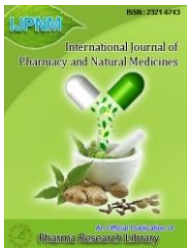

International Journal of Pharmacy and Natural Medicines
Journal Home Page: https://www.pharmaresearchlibrary.com/ijpnm
CODEN (USA): IJPNRC | ISSN: 2321-6743 | Publisher: Pharma Research Library
Int. J. Pharm. Natural Med., 2024, 12(1): 22-25.
DOI: https://doi.org/10.30904/j.ijpnm.2024.4659

In-vitro Anti-arthritic Activity of Leaves of *Momordica Charantia* Linn.,

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A B S T R A C T
The present study is aimed to evaluate the anti-arthritic activity of ethanolic extract of *Momordica charantia* leaves (EEML) by two *in-vitro* models *i.e.* human red blood cell (HRBC) membrane stabilization and inhibition of protein denaturation. The standard drug was diclofenac sodium. The results of both models showed concentration dependent inhibition of protein (egg albumin) denaturation as well as stabilization towards HRBC membrane. On the basis of present findings, it can be concluded that EEML showed anti-arthritic activity due to presence of phytocompounds such as flavonoids, alkaloids, tannins etc.
Keywords: Anti-arthritic activity, *Momordica charantia*, Karela, HRBC.

A R T I C L E I N F O

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Article History:
Received 22 Feb 2024
Revised 12 Mar 2024
Accepted 16 April 2024
Published 05 May 2024

Citation: *P. Gobala Krishnan, et al. In-vitro Anti-arthritic Activity of Leaves of Momordica Charantia Linn., Int. J. Pharm. Natural Med., 2024, 12(1): 22-25.*

C o n t e n t s

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease with chronic inflammation characterized by hyperplasia of synovial cells and angiogenesis in affected joints, which ultimately leads to the destruction of cartilage and bone. RA is a long lasting disease that can affect joints in any part of the body, most commonly the hands, wrists, and knees. RA affects three times more women than men [1-2]. RA is believed to be a T lymphocyte driven disease in which a sudden influx of T cells into the affected joint(s) is followed by an increased number of fibroblasts and macrophages, drawn by the release of cytokines, particularly interleukin-1(IL-1) and tumor necrosis factor alpha (TNF-α). This cytokine release and subsequent migration of cells is thought to be responsible for the chronic inflammation and characteristic destructive alteration in rheumatoid joints [3]. Momordica charantia (Family: Cucurbitaceae), is commonly known as bitter gourd or bitter melon in English and karela in Hindi [4]. It is a climber, widely cultivated as food in Asia, Africa and South America. It is also found all over India and cultivated up to an altitude of 1500m. The word Momordica is derived from the Latin word Mordeo which means to bite and the species name is derived from Greek word and it means beautiful flower [5]. M. charantia is very useful as antidiabetic, anti-inflammatory, antioxidant, antitumor, antiulcer, hypoglycemic, immunostimulant etc [6]. Our research group has already reported the anti-arthritic activity of fruit of M. charantia [7]. The root of M. charantia is useful in arthritis [8-10]. On the basis of

traditionally use; we have selected leaf part of M. charantia for the present study.

2. Materials & Methods:

Collection and authentication of plant material:

The leaves of Momordica charantia Linn., was collected from Tirupati. The plant material was identified as leaf of Momordica charantia Linn., and authenticated by Dr. K. Mahavachetty, Asst. Professor, Dept. of Botany, S V University.

Preparation of extracts

Extraction is the preliminary step involved in the phytochemical studies. It brings out the metabolites into the extracting solvent depends upon its polarity.

Extraction

The dried coarsely powdered leaves of Momordica charantia Linn., was first extract with n-Hexane (60-70Oc) in soxhlet apparatus and then with solvents of increasing polarity like Chloroform, Ethyl acetate and Ethanol at 60-70oC. Each extract was concentrated using rotary vacuum evaporator. The percentage yield, colour and consistency of these extracts were record and proceeded for further detailed phytochemical and pharmacological screening.

Phytochemical screening

Preliminary photochemical screening of EEML was carried out by previous established procedures.

In-vitro anti-arthritic activity

The following models have been performed

Human red blood cell (HRBC) membrane stabilization method [11]

Preparation of reagents

2 gm dextrose, 0.8 gm sodium citrate, 0.05 gm citric acid and 0.42 gm sodium chloride were dissolved in distilled water. The final volume was made up to 100 ml with distilled water. This mixture was used as Alsevers solution. Hypotonic saline was prepared by dissolving 0.36 gm of sodium chloride in 100 ml of distilled water. Isotonic saline was prepared by dissolving 0.85gm of sodium chloride in 100 ml of distilled water. 2.38 gm disodium hydrogen phosphate, 0.19gm of potassium dihydrogen phosphate and 8gm of sodium chloride were dissolved in 100 ml of distilled water. This was served as phosphate buffer (pH 7.4, 0.15 M).

Preparation of suspension (10% v/v) of human red blood cell (HRBC)

The blood was collected from healthy human volunteer who had not taken any NSAID'S for 2 weeks prior to the experiment and was mixed with equal volume of sterilized Alsevers solution. This blood solution was centrifuged at 3000 rpm and the packed cells were separated. The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline. This HRBC suspension was used for the study.

Assay of membrane stabilizing activity:

The assay mixtures contains 1ml of phosphate buffer, 2 ml of hypo saline and 0.5 ml of HRBC suspension and 0.5 ml different concentrations of extract, reference sample and control were separately mixed. 1ml of phosphate buffer, 2ml of hypotonic saline, 0.5ml of plant extract of various concentration (100, 200, 400, 800 and 1600 µg/ml) and 0.5ml of 10% w/v human red blood cells were used as test solution. 1ml of phosphate buffer and 2ml of water and 0.5ml of 10%w/v human red blood cells in isotonic saline were served as test control.1ml of phosphate buffer, 2ml of hypotonic saline, 0.5ml of standard drug (Diclofenac sodium) of various concentration (100, 200, 400, 800 and 1600µg/ml) and 0.5ml of 10% w/v human red blood cells were taken as standard solution. All the assay mixtures were incubated at 37ºc for 30 min. and centrifuged at 3000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560nm. The percentage hemolysis was estimated by assuming the hemolysis produced in control as 100%. [15,16] The percentage of HRBC membrane stabilization or protection was calculated by using the following formula-

Percentage protection: - 100- [(optical density sample/optical density control) × 100]

Inhibition of protein denaturation method: [12]

2ml of egg albumin (from fresh hen's egg), 28 ml of phosphate buffer (PBS, pH 6.4) and 20ml distilled water were used as control solution (50 ml). 2ml of egg albumin, 28 ml of phosphate buffer and various concentrations of standard drug (Diclofenac sodium) (10, 50, 100, 200, 400, 800, 1000 and 2000µg/ml) were served as standard drug solution (50 ml). 2ml of egg albumin, 28 ml of phosphate buffer and various concentrations of plant extract (10, 50, 100, 200, 400, 800, 1000 and 2000 µg/ml) were taken as test solution (50 ml). All of the above solutions were adjusted to pH, 6.4 using a small amount of 1N HCl. The samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling, the absorbance of the above solutions was measured using UV-Visible spectrophotometer at 660nm and their viscosity was determined by using Ostwald viscometer. The percentage inhibition of protein denaturation was calculated using the following formula-

Percentage inhibition = (Vt/Vc-1) x 100

Where, Vt = absorbance of test sample, Vc = absorbance of control.

3. Results and Discussion

Table.1 Qualitative phytochemical analysis

S.No	Phytoconstituent	Powdered drug	n-Hexane	Chloroform	Ethyl acetate	Ethanol	Aqueous
1.	Glycosides	+	+	-	+	+	+

2.	Steroids	+	+	+	+	+	+
3.	Carbohydrate	+	-	-	+	+	+
4.	Alkaloids	+	+	+	+	+	+
5.	Phenolic Compound	+	+	+	+	+	-
6.	Flavonoids	+	+	+	+	+	+
8.	Tannins	-	-	-	+	-	-
9.	Terpenoids	+	+	+	+	+	+
10.	Saponins	+	+	+	+	+	+
11.	Resins	-	-	-	-	-	-

Note: +Presence, - Absence.

Pharmacological Studies

In-vitro arthritic activity protein denaturation method

Table.2. Percentage inhibition of extracts-protein denaturation method

DRUGS	% INHIBITION AT VARIOUS CONCENTRATIONS				
	100µg/ml	200µg/ml	400µg/ml	800µg/ml	1000µg/ml
Diclofenac Sodium	24.15	32.50	41.87	60.23	72.13
n-Hexane	13.54	17.84	24.45	28.27	36.33
Chloroform	4.81	9.50	18.90	26.61	35.18
Ethyl acetate	16.81	21.54	37.73	41.95	48.15
Ethanol	21.25	28.12	42.51	57.17	63.75
Aqueous	2.84	5.97	15.70	23.33	31.35

Membrane Stabilization Method

Table.3. Percentage membrane stabilization of extracts

DRUGS	% Membrane stabilization				
	25µg/ml	50µg/ml	100µg/ml	200µg/ml	400µg/ml
Diclofenac sodium	65.09	71.63	75.93	83.26	87.51
n-Hexane	19.65	24.53	31.76	37.19	45.02
Chloroform	18.27	23.73	25.64	34.02	38.27
Ethyl acetate	22.43	28.39	37.44	53.93	61.82
Ethanol	38.08	42.18	49.29	67.01	72.65
Aqueous	15.37	19.72	27.49	37.29	39.58

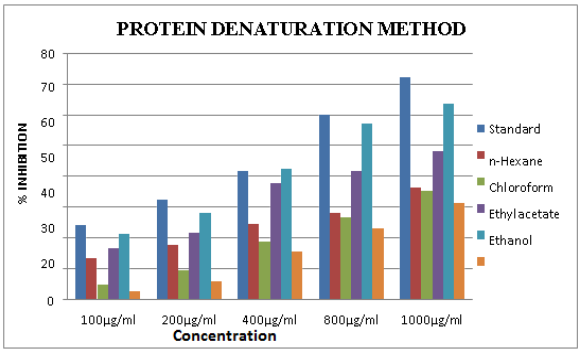

Fig.1. Graphical Data of Percentage Inhibition

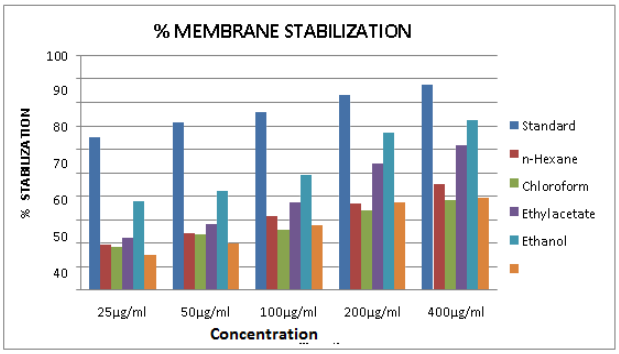

Fig.2. Graphical Data of Membrane Stabilization

The percentage inhibition of protein denaturation assay by n-Hexane, Chloroform, Ethyl acetate, Ethanol and aqueous extracts was found to be 36.33,35.18, 48.15, 63.75 and 31.35 respectively at maximum concentration 1000µg/ml. Percentage inhibition of Diclofenac sodium (Standard) was found to be 72.13. The results indicated that ethanolic extract showed the maximum percentage inhibition which is compared with the standard.

The percentage of membrane stabilization by n-Hexane, chloroform, ethyl acetate, ethanol and aqueous extracts was found to be 45.02, 38.27, 61.82, 72.65 and 39.58 respectively at maximum concentration of 400µg/ml. Percentage membrane stabilization of Diclofenac sodium was found to be 87.51. The results indicated that ethanolic extract showed the maximum percentage membrane stabilization which is compared with the standard. Both inhibition of protein denaturation assay and membrane

stabilization method indicated that of all the extracts, the ethanolic extract showed the maximum inhibition and stabilization activity. This correlates with the findings of the phytochemical study where the ethanol extract showed the presence of most of the phytoconstituents.

4. Conclusion

Pharmacognostic investigations

Exploration into the pharmacognostic characteristics of Momordica charantia Linn. leaves was undertaken, revealing distinctive features utilized for species differentiation.

Phytochemical analysis

Powdered leaves underwent successive extraction using n-hexane, chloroform, ethyl acetate, ethanol, and aqueous solvents employing a Soxhlet apparatus. Preliminary phytochemical screenings conducted on the powdered leaves and respective extracts unveiled the presence of flavonoids, saponins, alkaloids, triterpenoids, phenolic compounds, steroids, among others.

Pharmacological examinations

In-Vitro Investigations

Each extract underwent in vitro anti-arthritic studies to identify the most bioactive extract. Notably, the ethanolic extract exhibited the highest inhibition of protein denaturation and membrane stabilization activity, thus warranting its selection for in vivo studies.

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