Plant Archives Vol. 21, No. 2, 2021 pp. 290–294 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no2.045

IN VITRO ANTI-ARTHRITIC ACTIVITY OF DIFFERENT EXTRACTS OF *CASCABELA THEVETIA (THEVETIA PERUVIANA)* LEAVES

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(Date of Receiving : 22-02-2021; Date of Acceptance : 29-05-2021)

ABSTRACT

Cascabela thevetia (Thevetia peruviana) giant milkweed, is known for its pharmacological importance for centuries. The chemical constituents of *Thevetia peruviana* leaves are amyrin, amyrin acetate, β-sitosterol, urosolic acid, cardenolides, calotropin. The anti-arthritic activity of *Thevetia peruviana* leaves in comparison with Diclofenac was evaluated through *in-vitro* studies. *In-vitro* studies revealed anti-arthritic effects ($p< 0.05$) of crude extracts of aqueous, methanolic, ethanolic and chloroform of *Thevetia peruviana* leaves on bovine serum albumin as evident for % inhibition protein denaturation. Different extracts of *Thevetia peruviana* leaves were found to possess *in-vitro* anti-arthritic activity against BSA, using diclofenac as the reference standard which are Dose-dependent (100µg/ml, 250µg/ml, 500µg/ml). Chloroform extract has shown better activity comparing the other extracts.

Keywords: *Cascabela thevetia, Thevetia peruviana*, Bovine serum albumin, Diclofenac, anti-arthritic activity.

INTRODUCTION

Classical usage of medicine is cognized as a way to consider about the forthcoming future of medicines. As herbal medicines are cost effective and have high safety index got wide biological and medicinal values which is enhancing the desire and is used as genesis of basic health care in both progressed and also the non-progressed countries (UK Essays. (November 2015). Introduction To Medicinal Plants Biology Essay; Kamboj, V.P. 2000) Inflammation is a shielding feedback to tissue injury which include a multiplex sequence of enzyme activation, release/liberation of mediators, fluid extravasations, cell migration, breakdown and repair of tissue (Vane, 1971). It is distinguished by signs like rubor (redness), swelling(tumor), pain (dolar), color (heat),stiffness of joint and function laesa (loss of joint function). It is correlated with membrane modification, elevation in vascular permeability and denaturation of proteins (Umapathy *et al.,* 2010). Arthritis is the most common chronic inflammation andpremier reason of disability globally. There are several types of arthritis and correlating conditions, among which rheumatoid arthritis and osteoarthritis are the two crucial ones. Mainly joint diseases affect synovial joints. Symptoms will be different from one type to other. Few may have mild symptoms and some with powerful symptoms. Some of the clinical manifestations include, Pain, rash, cramping, Edema of Joints, Rigidity, Constipation, Stuffy or runny nose, Tenderness, Redness, Warmth, Discomfort when Standing or Walking, Loss of Flexibility, Limping, Bone Spurs, Fatigue (feeling tired) (Harsh Mohan, 2010; Iain, *et al.,* 2011; Joseph, 2008) The body's natural-bornimmune systems are a part of the problem in rheumatoid arthritis. Due to some unspecified reason, instead of protecting, the immune system starts striking few parts of the body (Joseph, 2008). The modern therapy of arthritis includes reduction of this associated pain and inflammation using non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARD), corticosteroids, immunosuppressant and newer biological agents such as TNF-α and monoclonal antibodies Due to many adverse reactions of the NSAIDs and disease modifying anti-rheumatic drugs, the patients tend to show interest for other treatments that are safe, potent, cost effective and less toxic. Therefore, complementary and alternative medicines are generally preferred by such patients (Lipincottwiliams and Wilkins, 2015).

Plants have been a rich source of medicines which are potent as a well as devoid of adverse effects. *Thevetia peruviana* is a synonym of *Cascabela thevetia* belonging to the family of Apocynaceae and is commonly known as Yellow oleander. It is a small everlasting tree (3-4 m high) cultivated as an decorative plant in tropical & subtropical regions of the world, including India and other few countries (Kumar *et al.,* 2017). It has been reported to possess anti-microbial,anti-fungal,piscicidal,anti-spermatogenic,anti-diarrhoeal,anti-termite and many other properties.(Kishan *et*

al., 2012)Our aim is to study the anti-arthritic activity of various extracts of *T. peruviana* leaves.

Pathogenesis of rheumatoid arthritis (Iain *et al.,* 2011; Joseph T Dipiro. 2008; Rubin *et al.,*2005)

- In response to exposure of any foreign material (antigen) in a genetically predisposed individual (HLA-DR), CD4+T-Cell are activated.
- Cytokines(TNF α, IL-1 and IL-6) are elaborated by these cells.
- Cytokines then activate B lymphocytes, macrophages& endothelial cells.
- The activated B-cells release anti IgG antibody(Ab) (IgM antibody) –termed as rheumatoid factor
- It forms complexes which cause inflammatory damage to the synovium, small blood vessels and collagen.
- Activation of endothelial cell activate inflammatory cells by expressing adhesion molecules.
- Macrophages when stimulated releases more cytokines causing damage to joint tissue and formation of pannus.
- Bone and cartilage damage along with fibrosis and ankylosis results in the deformation of joints.

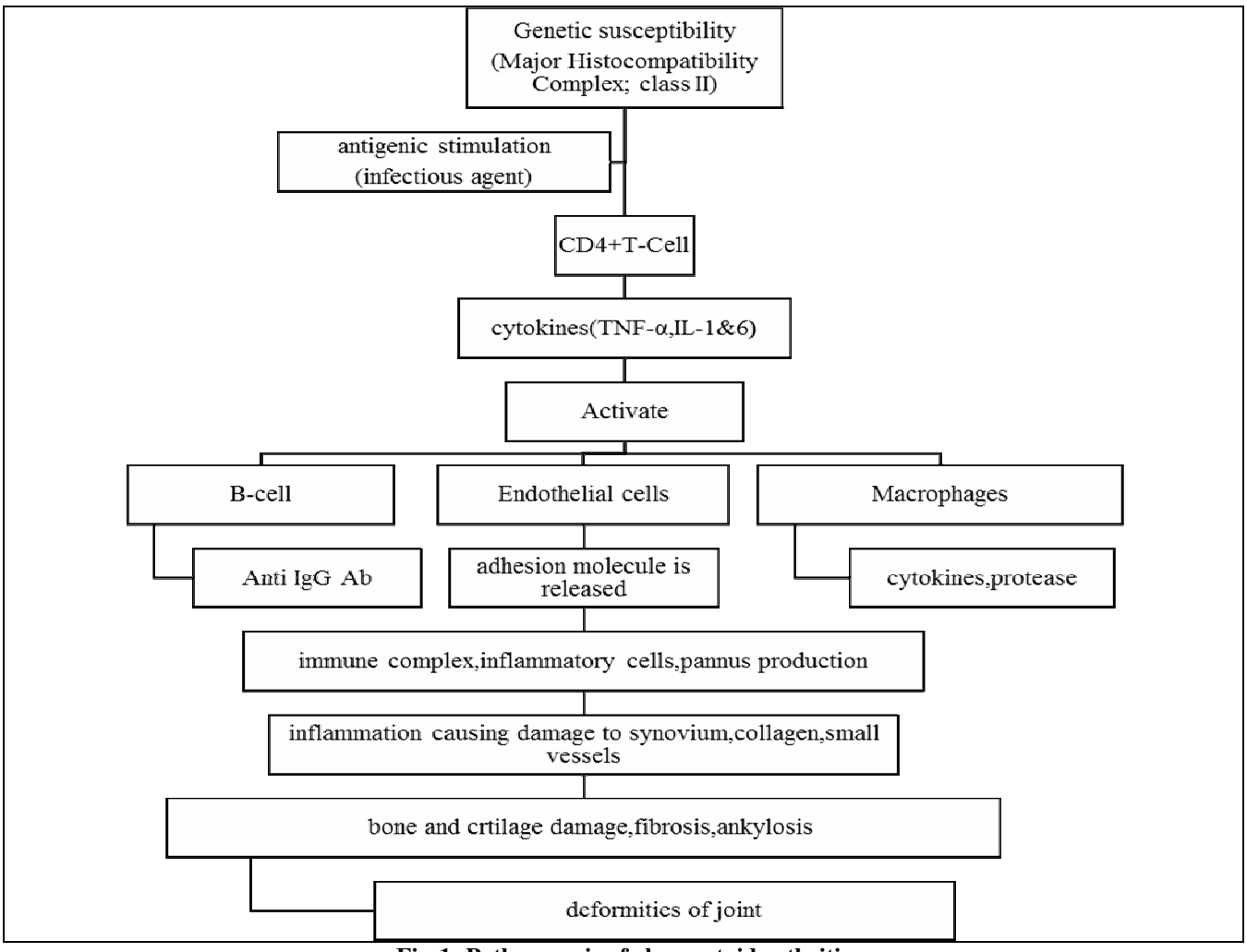


Fig-1: Pathogenesis of rheumatoid arthritis

MATERIALS AND METHODS

Collection of plant materials:

The leaves of Cascabela thevetia (*Thevetia peruviana)* were collected from the local areas of Razvichaman, Karimnagar District, Telangana in January 2020. The *T. peruviana* species were voucher specimen, has been identified by Botanical Survey of India, Deccan Regional Centre, Hyderabad, Telangana, India. (BSI/DRC/2020-21/Tech./Identification/50) The leaves of *Thevetia peruviana* were gathered, washed and dried at room temperature. The dried plant stuff was minced into powder. The powder was exposed to various studies for which materials and methods presented below.

Physico-chemical analysis:

Proximate analysis (Bhargava *et al.,*2013):

Determination of Ash values:

Total ash:

2 gm of precisely weighed powdered drug was placed in a tarred silica dish and was burned at a temperature of 450°C until freed from carbon. The sample was cooled and while later weighed. The degree of ash was assessed by comparing to the air-dried drug.

Acid insoluble ash:

The acquired total ash was bubbled for 5 min. with 25 ml of dilute HCl. The insoluble stuff was gathered on an ash-less filter paper and cleaned with heated water later ignited to obtain steady weight. The degree of acid insoluble ash was determined by comparing to the air-dried drug.

Water soluble ash:

To the total ash 25 ml water was added and bubbled for 5 minutes. The insoluble stuff was gathered on an ash less filter paper, cleaned with heated water and burned in a crucible for 15 minutes at a temperature of not more than 450°C. The weight obtained from this residue was deducted from the weight of total ash. The amount of water-soluble ash with reference to that of air-dried drug was determined.

Determination of extractive values:

Alcohol soluble extractive:

5% solution in methanol was made by adding 5gm of powdered drug with 100ml of methanol in a conical flask. After a while the flask was closed with the help of the cotton plug. The blend was shaken after normal time period without contacting the solution on to the cotton plug. The blend was kept for 24hrs and was filtered out with the help of the Whatman filter paper. The solid on the upper side was discarded and filtrate was gathered. Void evaporating dish was brought and weighted and placing 25ml of 5% solution of drug was heated until the damp mass is formed. Evaporating dish was cooled and weighed. Extractive value was directly determined by assessing the difference between evaporating dish containing the damp mass to the empty evaporating dish.

Water soluble extractive:

5% solution in water was made by mixing 100ml of water to the 5g of powdered drug in a conical flask. The flask was stoppered with the help of the cotton plug, shaken after regular time period without contacting the solution on to the cotton plug, kept aside for 24hrs.(with regular shaking) and was filtered using Whatman filter paper. The solidon the upper side was discarded and filtrate was gathered. Void evaporating dish was brought and weighted and placing 25ml of 5% solution of drug was heated until the damp mass is formed. Evaporating dish was cooled and weighed. Extractive value was directly determined by assessing the difference between evaporating dish containing the damp mass to the empty evaporating dish.

Fluorescence analysis (Kokoski *et al.,* 1958):

A little amount of dried powder and add 1-2 drops of freshly prepared reagent solution was set on a grease free clean microscopic slide, fused by tilting the slide gently and was set inside the UV chamber and analyze the colour in short (254 nm) and long (365 nm)visible light, Ultra Violet radiations. The chrome seen by the application of various reagents in varied radiations was noted.

Preparation of extracts:

Taking 100g of coarse *Thevetia peruviana* powder packed and continuous hot Soxhlet extraction was performed using various solvents (95% ethanol, methanol, chloroform and water).The extract was filtered and concentrated operating rotary vacuum evaporator. Percentage yield was determined and UV absorbance analysis was performed for three different wavelengths.

Phytochemical screening:

Various phytochemical tests were performed like test for alkaloids, anthraquionones, flavonoids, coumarins, phenols, saponins, steroids, terpenoids, tannins, amino acid& protein and carbohydrates as per the standard reference books Kokate, C.K. 2008; Kokate, C.K., A.L., 2006, Harborne, Khandelwal, K.R. 2002.

Preparation of reagents:

1%mM Bovine Serum Albumin solution (BSA):

In 100ml of phosphate buffer (0.2M, pH-7.4) add 1 g of Bovine serum albumin and dissolve it clearly.

Method:

Protein Inhibition Denaturation Assay:

Test solution (0.5ml):

0.45ml of BSA (1%mM) phosphate solution + 0.05ml of test drug solution of various con. i.e., 100 µg/ml, 250 µg/ml and 500 µg/ml)

Test control solution (0.5ml):

0.45ml of BSA (1%m Mphosphate solution) + 0.05ml of distilled water.

Product control (0.5ml):

0.45ml of phosphate buffer (0.2M, pH-7.4)+ 0.05ml of test drug solution.

Standard solution (0.5ml):

0.45ml of Bovine Serum Albumin (BSA) (1%m Mphosphate solution) + 0.05ml of Diclofenac sodium of various concentrations.

Test arrangements and standard (Diclofenac) 1ml each of varying concentrations was fused with 1 ml of 1%mM solution of albumin in phosphate buffer (0.2M, pH 7.4) excluding drug for control and incubated for 15min at 27± 1°C in Biological Oxygen Demand incubator. Later placing at 60±1°C in water bath for 10 min denaturation was induced. Finally, after cooling turbidity was calculated using UV visible spectrophotometer, (ANALYTICA TL64) at 660nm. Triplicate results for each concentration were determined and average was taken. (Gondkar *et al.,* 2013)

Percentage inhibition of protein denaturation was determined utilizing the accompanying equation:

$$\text{Percentage Inhibition} = 100 - ((V_c / V_t) - 1)$$

V_c & V_t=Mean absorbance value of control and test

RESULTS

Proximate Analysis:

Table 1: Results of proximate analysis

Parameters	Values(%w/w)
Total ash	3.51
Acid insoluble ash	1.62
Water soluble ash	1.30
Alcohol soluble extractive	8.30
Water soluble extractive	4.80

Fluorescence Analysis:

Table 2 : Fluorescence analysis of *T. peruviana* leaf powder in different solvents

Treatment	Under visible light	Under UV light
Powdered drug	Green	Green
Powder + 1N NaOH (aqueous)	Yellow	Green
Powder + 1N NaOH (ethanolic)	Green	Orange
Powder + 1N HCl	Yellow	Greenish red
Powder + 50% HNO_3	Orange	Red

Phytochemical screening:

Table 3: Phytochemical analysis of different extracts.

S.NO.	Phytoconstituents	Ethanolic ext.	Methanolic ext.	Chloroform ext.	Aqueous ext.
A	Alkaloids	+	+	+	-
B	Amino acids	-	-	-	-
C	Anthraquinones	+	-	-	+
D	Coumarins	-	-	-	-
E	Carbohydrates	-	-	-	+
F	Flavonoids	+	+	+	+
G	Phenolics	+	+	+	-
H	Proteins	-	+	-	-
I	Steroids	+	+	+	+
J	Saponins	+	+	+	+
K	Tannins	+	+	+	-
L	Terpenoids	-	+	+	-

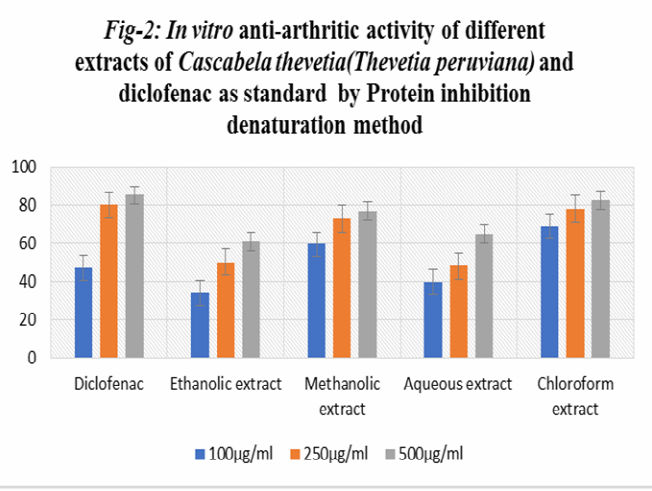
In-vitro anti-arthritic activity:

The rate hindrance of protein denaturation assay by Chloroform, aqueous, methanolic and Ethanolic extracts was discovered to be 82.86±2.18%, 65.00±2.97%, 77.14±3.78% and61.05±4.23% respectively. At greatest convergence of 500µg/ml. percentage inhibition of Diclofenac (Standard) was determined to be 85.29±1.65%. Chloroform extract showed the greatest rate hindrance when contrasted to standard. Dose dependent response has been found from all the extracts. Phytoconstituents like steroids, alkaloids, tannins, saponins and flavonoids might be the reason for the effect. Further studies are focused on isolation of phytoconstituents which is answerable for the activity and *in-vivo* models to be studied.

Table 4: Percentage inhibition of different extracts of *Cascabela thevetia* (*T. peruviana)*

Compound	100µg/ml	250µg/ml	500µg/ml
Diclofenac	47.21±1.65**	80.29±1.65***	85.29±1.65***
Methanolic extract	59.52±2.08**	72.86±3.30***	77.14±3.78***
Aqueous extract	39.52±2.08**	48.00±3.78***	65.00±2.97***
Chloroform extract	69.05±3.33***	78.10±2.90***	82.86±2.18***
Ethanolic extract	33.81±2.08**	50.24±2.90***	61.05±4.23***

Results are expressed as mean ± SEM (n=3); Significant differences with respect to standard was evaluated by one-way ANOVA, Dunnett's t-test. *P<0.05, **P<0.01, ***P<0.001.



CONCLUSION

Significant anti-arthritic property was found by performing *in vitro* studies on leaves of *Thevetia peruviana*. Chloroform extract has shown more anti-arthritic activity than ethanolic, methanolic and aqueous extracts. The Activity might be because of the presence of phytoconstituents like alkaloids, tannins, steroids, flavonoids and terpenoids. *In-vivo* investigation should be done further by isolating phytoconstituents responsible for the activity.

REFERENCES

Bhargava, V.V.; Saluja, A.K. and Dholwani, K.K. (2013). Detection of Heavy Metal Contents and Proximate Analysis of roots of *Anogeissus latifolia, Journal of Pharmacognosy and Phytochemistry,* 1(6): 61-660.

Gondkar, A.S.; Deshmukh, V.K. and Chaudhari, S.R. (2013). Synthesis, characterization and *in-vitro* anti-inflammatory activity of some substituted 1,2,3,4 tetrahydropyrimidine derivatives. *Drug Invention Today,* 5(3): 175-181.

Harsh, M. (2010). Text book of pathology, 6th ed. Jaypee publications.

Iain, B.; McInnes and Georg Schett (2011). The pathogenesis of rheumatoid arthritis, *The New England Journal of Medicine*, 365: 2205-2219.

Introduction to medicinal plants biology essay. Uk essay, 2015 March. Ht *Thevetia peruviana* s://www.ukessays. com/essays/biology/introduction-to-medicinal-plants-biology-essay.ph. (2016 Nov 21).

Joseph, T.D. *Pharmacotherapy: A pathophysiological approach*. 7th ed, (2008).

Kamboj, V.P. (2000). Herbal medicine, *Current Science*, 78(1): 35-51.

Kishan, S.; Kumar, A.K.; Vimlesh, M.; Mubeen, U.S. and Alok, S. (2012). A review on: *Thevetia peruviana. International Research Journal of Pharmacy,* 3(4): 74-77.

Kokoski, J.; Kokoski, R. and Salma, F.J. (1958). Fluorescence of powdered vegetable drugs under ultraviolet radiation, *Journal of the American Pharmacists Association*, 47: 715-717.

Kumar, A.; Tyagi, V.; Rathi, B.; Priyanka and Manisha (2017). Chronological Review on Phytochemical, Antioxidant, Antimicrobial and Clinical studies on Biodiesel Yielding Good Luck Tree (*Thevetia peruviana*), *International Journal of Pure & Applied Bioscience,* 5(6): 1499-1514.

Lipincottwiliams and Wilkins (2015). *Pharmacology*, 6th ed. Wolters kluwer (India) Pvt Ltd, pp: 457-458.

Rubin, E.; Gorstein, Rubin, R.; Schwarting and Strayer (2005). *Rubin's Pathology clinicopathologic foundation of medicine*, Rheumatoid arthritis. 4th ed.

Umapathy, E.; Ndebia, E.J.; Meeme, A.; Adam, B.; Menziura, P.; Nkeh-Chungag, B.N. and Iputo, J.E. (2010). An experimental evaluation of *Albucasetosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation, *Journal of Medicinal Plants Research,* 4(5): 789-795.

Vane, J.R. (1971). Inhibition of prostaglandins synthesis as a mechanism of action for aspirin like drugs, *Nature*, 231(2): 232-235.