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In-Vitro & In-Vivo Evaluation of Anti-Inflammatory and Anti-Oxidant Activity on Hyptis Suoveolens (Seeds) & Tribulus Terrestris (Fruit)

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Abstract: ***Objective: The main objective is to study on anti-inflammatory and anti-oxidant activity on various animal models by using In-vitro and In-vivo anti-inflammatory activity on chloroformic extract of Hyptis Suoveolens seeds and N-hexane extract of Tribulus Terrestris fruit. The study confirmed the calm of the drug use in reducing inflammation. The compounds responsible for reducing inflammation are in progress of identification. Materials and methods: Hyptissuaveolens seeds and Tribulusterrestris fruit were extracted and subjected to protein denaturation using eggalbumin and gelatin zymography studies. The extract was screened for anti-inflammatory activity in albino rats using acute carrageenan induced paw edema in rats, egg albumin induced inflammation and hot-plate test. Results: The extract shows the presence of chemical compounds with chloroform, n-hexane and methanol and in-vivo anti-inflammatory activity was done by the acute models. Conclusion: The extract of hyptissuaveolens seeds and tribulusterrestris fruit showed anti-inflammatory activity in acute and chronic study in albino rats.***

Keywords: Hyptissuaveolens seeds, Tribulusterrestris fruit, diclofenac sodium, albino rats

1. Introduction

Inflammation is a defensive response triggered when the body is threatened by for example pathogens, damaged cells or irritants. These responses are essential for humans in combating infections and for promoting healing and restoration to normal function in the event of injury. Unfortunately, these defensive responses can occasionally go wrong, leading to different inflammatory diseases.

Inflammatory diseases include rheumatoid arthritis, atherosclerosis, Alzheimer's, asthma, psoriasis, multiple sclerosis, and inflammatory bowel disease, and many of these inflammatory diseases are becoming common throughout the world. According to World Health Organization (WHO) estimates, about 235 million people suffer from asthma and it is the most common chronic disease among children. Approximately 0.3-1.0% of the general population is affected of rheumatoid arthritis. It has been estimated that rheumatoid arthritis affects approximately 1.5 million people in the United States and the prevalence in Norway is estimated to be about 0.5%. Like other inflammatory diseases, asthma and rheumatic conditions continue to be a large and growing public health problem.

The three major groups of drugs used in treatment of inflammatory diseases are corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), and disease-modifying anti-rheumatoid drugs (DMARDs), which include biological agents. These drugs are widely used and are effective in treating many inflammatory diseases. Corticosteroids also play a major role in the therapy of organ transplantation because of their anti-inflammatory and immunosuppressive effects. Unfortunately, they are also associated with several serious side effects, while the biological agents are expensive in use. Corticosteroids are well known for causing Cushing's syndrome, in addition to other adverse effects such as hyperglycemia, increased susceptibility to infection, psychiatric disturbances, etc.

Long term use of NSAIDs causes gastrointestinal ulceration and bleeding and platelet dysfunction. Inflammation is a complex process involving a multifunctional network of chemical signs to mediate the action. The primary anti-inflammatory targets include cyclo-oxygenase (COX)-1 and 2 enzymes, cytokines such as tumor necrosis factor (TNF)-α and interleukins (IL-1β, IL-6), and transcription factor as nuclear factor (NF)-κB and several more. Resolution of inflammation. The transcription factor NF-κB is a main regulator of TNF-α and ILs have been identified to play a central role in the pathogenesis of much inflammation disease, especially asthma and rheumatoid arthritis. TNF-α and ILs are intercellular signal proteins released by immune cells, and have many functions in promotion and the expression of several genes involved in the activation of inflammation. NF-κB has been described to have a major role in pathogenesis of inflammatory bowel disease and also of rheumatic diseases. It is well established that the excessive production of pro-inflammatory mediatorsis implicated in4several inflammatory diseases. Therefore, inhibition of the overproduction of these mediators is a crucial, exciting target in treatment of these conditions.

There are two main strategies for research in the area of natural products; older-and modern strategy. In the older strategy, the chemistry of compounds is in focus and selection of natural sources is based mainly on ethno-pharmacological information as well as traditional uses. Isolation and identification of compounds are performed before biological activity testing (primarily invivo).

The modern strategy, so-called bioactivity-guided isolation is, as the name indicates, more focused on bioactivity. Biological assay (mainly invitro) are used to target in isolation of bioactive compounds. Selection of organisms is based on ethnopharmacological information and traditional use, but might also be randomly selected. In modern strategy natural marine sources are particularly utilized.

Antioxidants:

A substance that protects cells from the damage caused by free radicals (unstable molecules made by the process of oxidation during normal metabolism). Free radicals may play a part in cancer, heart disease, stroke, and other diseases of aging. Antioxidants include beta-carotene, lycopene, vitamins A, C, and E, and other natural and manufactured substances.

Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Diets high in vegetables and fruits, which are good sources of antioxidants, have been found to be healthy; however, research has not shown antioxidant supplements to be beneficial in preventing diseases. Examples of antioxidants include vitamins C and E, selenium, and carotenoids, such as beta-carotene, lycopene, lutein, and zeaxanthin.

2. Materials and Method

In-vitro studies:

Plant Material: For the present investigation, seeds of hyptis suaveolens and tribulus terrestris fruit were purchased from the local market in Guntur, Andhra Pradesh. The seeds were identified and authenticated by a botanist in Acharya Nagarjuna University and the specimen sample is deposited in the Pharmacognosy division, SIMS College of Pharmacy, Guntur.

Preparation of Extract: The procedure of extraction used for hyptis suaveolens and tribulus terrestris. The seeds of hyptis suaveolens and the fruit of tribulus terrestris are powdered and was soaked in water, ethanol, methanol, chloroform, ethyl acetate and N-hexane and it allowed to stand for three days (72hrs), by shaking occasionally. The liquid is strained off and the solid residue (marc) is pressed (to recover as much as occluded solution). The obtained liquid is clarified by subsidence or filtration. In the preliminary phytochemical screening (Trease and Evan, 1959), the filtration of both plants were done by phytochemical test and gives positive results for alkaloids, carbohydrates, proteins and flavonoids and negative for steroids, glycosides and phenols. Hyptis suaveolens, tribulus terrestris and standard reference drug were dissolved in normal saline and administered orally to rats in dose volume of 1ml/kg body weight. The dilutions were prepared every time just prior to administration.

Chemicals: Carrageenan, egg albumin, N-hexane**,** Chloroform**,** Methanol**,** Agarose gel**,** Ph 6.4 buffer**,** Diclofenac Sodium**,** DPPH**,** DMSO**,** Ascorbic acid**,** Molisch reagent**,** Concentrated H2SO4**,** Alcoholic solution, Bendicts reagent**,** Feling's solution A&B**,** Millon's reagent**,** Copper sulphate solution**,** Ethanol**,** Potassium hydroxide pettete**,** Sudon red-4**,** Ferric chloride solution, Gelatin**,** Nacl**,** Lead acetate, Ammonium hydroxide solution, Magnesium metal**,** Hcl**,** NaoH**,** Acetic anhydride**,** Ammonia solution, Pyridine**,** Sodium nitroprusside**,** Mayer's reagent**,** Picric acid, Wangner's reagent**,** Dragendorff's reagent**,** Glacial acetic acid**,** Ninhydrin reagent**.** These chemicals were obtained from SIMS College Of Pharmacy, Acharya Nagarjuna University, Guntur.

Anti-Inflammatory Activity using Gelatin Zymogrphy (Detection of MMP-2 and MMP-9):
Clean electrophoresis apparatus in warm water and clean glass plates in Methanol. Set up plates. Large plate, then two spacers (apply little petroleum jelly on both sides of the Spacers) and small plate on top. Assemble plates into clamp and gently tighten screws. Do not over tighten, as this will crack the plates. Heat the agarose gel, pour between the two glass plates just to seal the bottom surface, leave it for 5-10 minutes. Prepare the resolving gel as given below.

Preparation of 10% resolving gel (10ml)

Regents	Volume
Acrylamide-Bisacrylamide	3.3ml
Resolving gel buffer stock	1.25ml
10% SDS	100µl
1.5% APS	500 µl
Gelatin	1ml
Water	3.8ml
Temed	10µl

Mix the appropriate resolving gel mixture and pipette between the glass plates avoiding bubbles. Fill plates about 80% way up leaving space for the stacking gel and comb. Overlay with a small amount of water to achieve a completely flat interface between resolving gel and stacking gel. Allow to set for about 45 minutes. While resolving gel is setting prepare the stacking gel.

Preparation of 5% stacking gel (10ml)

Regents	**Volume**
Acrylamide-Bisacrylamide	1.7ml
Stacking gel buffer	1.12ml
10% SDS	100µl
1.5% APS	500µl
Water	6.5µl
Temed	10µl

When resolving gel is set pour off the excess water and wash between the plates with distilled water. Pour stacking gel and insert comb avoiding bubbles. Allow to set for about 30 minutes. When stacking gel is set gently remove comb, wash the wells with distilled water and assemble gels onto the electrode/gasket section of the gel apparatus. Fill top and bottom of the tank with reservoir buffer i. e. upper tank with 100ml and lower tank with 150ml. Preparation of MMP samples: The MMP samples will be collected from the patients who have undergone Tonsilectomy. The collected tonsil samples will be minced using 1-2ml of extraction buffer. The minced sample along with extraction buffer is transferred to a falkan tube. Seal it properly and store at-20ºC. Before starting the experiment the stored samples are taken out from-20ºC, thaw it and centrifuge at 3000rpm for 10-15mins. Supernatant is used for further experiment.

In-vivo studies:

Experimental animals: Experiment was performed using wister albino rats (150-200g) of either sex, produced from the Mahaveera enterprises, Hyderabad (India). All the animals were maintained under controlled room temperature of 25 ± 1^0 C and 12hr/12hr light/dark chamber in a polypropylene cage. The animals were housed in colony

cages (three animals per cage) with free access to feed and water. Guidelines of Institute animal Ethics Committee were followed while using live animals. All the animals were acclimatized to the laboratory environment for 5 days before the experiment. Six animals per group comprising of three males and three females were used in each experiment, unless otherwise specified. The animals were fasted overnight just prior to the experiment but allowed free access to drinking water.

Acute toxicity study: The hyptis suaveolens and tribulus terrestris was administered orally in graded doses of 100, 150, 200 mg/kg to three different group of rats, while the control group receives vehicle (2ml/kg, p. o.) alone. All treated animals were closely observed for any abnormal or toxic manifestations and for mortality up to the end of 24h in each group to calculate LD50 by the method described by Weil (1952).

Anti-inflammatory activity:

Carrageenan induced paw edema in rat: 1% carrageenan solution (1 gram in 100ml water). Before inducing carrageenan, rats were fed orally by the prepared pure extract solution of 0.2mg for 14 to 21 days and the standard drugs of 0.4 mg was given before 2 days of inducing and control rat was remain salient for 14 to 21 days. After 14 to 21 days of feeding at final day rats were fasted for 24hrs. After fasting for 24hrs, prepared carrageenan was induced to rats at right hind paw up to the volume of 0.2-0.3 units at sub-plantar tissue after inducing the values were observed for every 30min, 1hr, 2hr, 3hr and 24hrs. The values were observed by plethysmograph or Vernier caliper.

Egg albumin induced inflammation: 10 ml normal saline solution was added to 2 grams of fresh egg albumin (0.1 ml, 20% in normal saline).

Before inducing egg albumin, rats were fed orally by the prepared pure extract solution of 0.2mg for 14 to 21 days and the standard drugs of 0.4 mg was given before 2 days of inducing and control rat was remain salient for 14 to 21 days. After 14 to 21 days of feeding at final day rats were fasted for 24hrs. After fasting for 24hrs, prepared egg albumin was induced to rats at right hind paw up to the volume of 0.2-0.3 units at sub-plantar tissue after inducing the values were observed for every 30min, 1hr, 2hr, 3hr and 24hrs. The values were observed by plethysmograph or Vernier caliper.

Hot-plate test:
Before testing, rats were fed orally by the prepared pure extract solution of 0.2mg for 14 to 21 days and the standard drugs of 0.4 mg was given before 2 days of inducing and control rat was remain salient for 14 to 21 days. Each female rat was kept in hot-plate having a constant temperature of 55+/-5 degree Celsius, normally animal show response in 6-8 sec and a cut off period is 15 sec is observed to avoid damage to the paw and response time was recorded as the time elapsed before the rat responded by licking of a hind paw. The values were observed for every 30min, 1hr and 2hrs, the values were recorded.

3. Results and Discussion

In-Vitro Studies:

Preliminary phytochemical screening of extract:
The result of different phytochemical screening of hyptis suveolens seeds and tribulus terrestris fruit showed the compounds are present like alkaloids, carbohydrates, proteins and flavonoids while steroids, glycosides and phenols components are showed as absent.

Solubility Test: Table: 1:

Solubility	Hyptis Suveolens	Tribulus Terrestris
Water	---	---
Ethanol	---	---
Methanol	---	---
Chloroform	+++	+++
Ethyl acetate	---	---
N-hexane	+++	+++

+ = positive,-= negative

Phytochemical Test: Table: 2:

Samples	Hyptis Suveolens	Tribulus Terrestris
1. Carbohydrates:		
• Molish's test	Positive [+++]	Positive [+++]
• Bendict's test	Positive [+]	Negative [---]
• Fehling's test	Negative [---]	Negative [---]
2. Proteins:		
• Millon's test	Positive [+]	Positive [+]
• Biuret test	Negative [---]	Negative [---]
3. Fats and fixed oils:		
• Spot test	Negative [---]	Negative [---]
• Saponification test	Negative [---]	Negative [---]
4. Volatile oils	Negative [---]	Negative [---]
5. Flavonoids	Positive [+++]	Positive [+++]
6. Tannins	Negative [---]	Negative [---]
7. Terpenoids	Negative [---]	Negative [---]
8. Saponins	Negative [---]	Negative [---]
9. Steroids	Negative [---]	Negative [---]
10. Phytosteroids:		
• Libermann-Burchard's test	Negative [---]	Negative [---]
11. Phlobatannins:	Negative [---]	Negative [---]
12. Glycosides:		
• Borntrager's test	Negative [---]	Negative [---]
• Legal's test	Negative [---]	Negative [---]
13. Coumarins	Positive [++]	Positive [+] Pale yellow
14. Alkaloids:		
• Mayer's test	Positive [+]	Positive [+]
• Wagner's test	Positive [+]	Positive [+]
• Dragendorff's test	Positive [+]	Positive [+]
15. Anthraquinones:	Negative [---]	Negative [---]
16. Anthracyanins:	Negative [---]	Negative [---]
17. Quinones:	Negative [---]	Negative [---]
18. Phenols:		
• Ferric chloride test	Negative [---]	Negative [---]
• Gelatin test	Negative [---]	Negative [---]
• Lead acetate test	Negative [---]	Negative [---]
• Alkaline reagent test	Positive [+]	Positive [+]
• Magnesium and Hcl reduction	Negative [---]	Negative [---]
19. Cardiac glycosides:	Negative [---]	Negative [---]
20. Ninhydrin [amino acid]:	Positive [++]	Negative [---]
21. Gum and Mucilage:	Positive [++]	Negative [---]

Determination of Albumin denaturation test:

Anti-inflammatory effect of hyptis suaveolens and tribulus terrestris on inhibition of protein denaturation using egg albumin:

The reaction mixture (5 ml) included egg albumin (0.2 ml), phosphate buffered saline 2.8 ml (pH6.4) and 2 ml of *Hyptissuaveolens* seed extract and diclofenac sodium at various concentrations (12.5, 25, 50, 100, 200, 400 and 800 µg/ml), respectively. Equal volume of double-distilled water served as control. The mixtures were incubated at 37±2 °C in a Biochemical oxygen demand (BOD) incubator for 15 min at 37°C and then heated at 70°C for 5 min. their absorbance was measured at 660nm. The percentage inhibition of protein denaturation was appraised using under mentioned formula:

% inhibition = (Abs control-Abs test sample / Abs control) * 100

Abs=Absorbance.

Hyptis Suoveolens: Table: 3:

Distilled water	Diclofenac sodium	Albumin	Ph
0.021 ppm	0.033 ppm	0.006 ppm	0.016

Sample	N-hexane	Methanol	Chloroform	Water
Sample-1	0.029 ppm	0.035 ppm	0.025 ppm	0.023 ppm
Sample-2	0.026 ppm	0.038 ppm	0.014 ppm	0.029 ppm
Sample-3	0.009 ppm	0.036 ppm	0.021 ppm	0.029 ppm

Table: 4: Absorbance value

% inhibition = (Abs control-Abs test sample / Abs control) * 100

Abs=Absorbance.

Sample	Absorbance
N-Hexane	- 31.42%
Methanol	- 72.85 %
Chloroform	4.76 %
Water	- 28.57 %

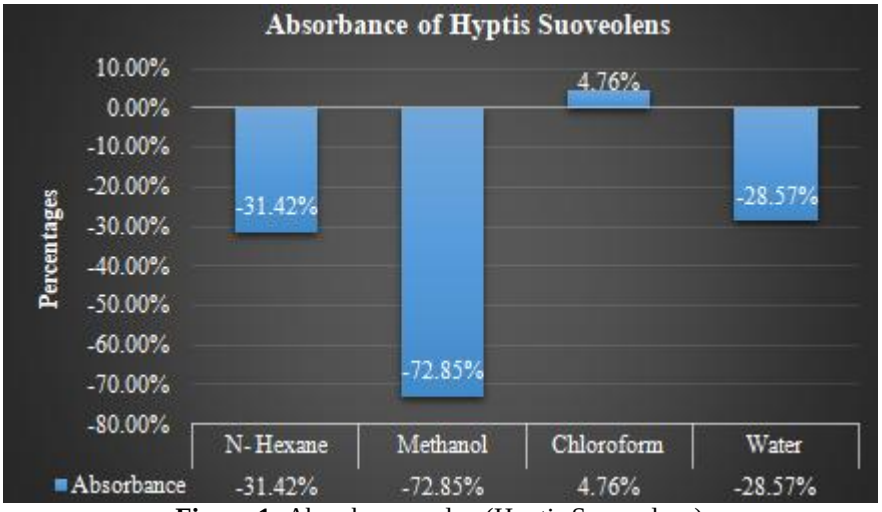


Figure 1: Absorbance value (Hyptis Suoveolens)

Tribulus Terrestris

Table 5

Distilled water	Diclofenac sodium	Albumin	Ph
0.021 ppm	0.033 ppm	0.006 ppm	0.016

Table: 6: Absorbance value

Sample	N-hexane	Methanol	Chloroform	Water
Sample-1	0.002 ppm	0.012 ppm	0.013 ppm	0.020 ppm
Sample-2	0.001 ppm	0.008 ppm	0.011 ppm	0.016 ppm
Sample-3	0.002 ppm	0.010 ppm	0.011 ppm	0.013 ppm

% inhibition = (Abs control-Abs test sample / Abs control) * 100

Abs=Absorbance.

Sample	Absorbance
N-Hexane	92.38%
Methanol	52.38 %
Chloroform	44.76 %
Water	22.38 %

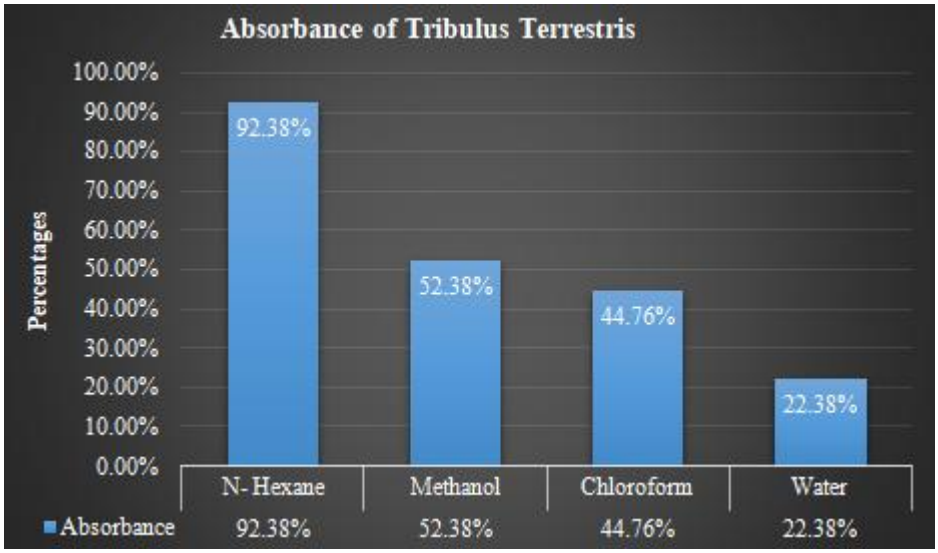


Figure 2: Absorbance value (Tribulus Terrestris)

Determination of in-vitro antioxidant activity:

Anti-inflammatory effect of hyptis suaveolens and tribulus terrestris on DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay:

The free radical scavenging potential of different extracts were determined according to the procedure of kulisic with some modifications. An aliquot of 50 µL of sample solution of various concentrations (25-400 µg/ml) were mixed with 950µL of methanolic solution of DPPH (3.4 mg/100 ml). The reaction mixture was incubated at 37°C for 1 hour in the dark. The free radical scavenging potential of the extracts were expressed as the disappearance of the initial purple color. The absorbance of the reaction mixture was recorded at 517 nm using colorimetry. Ascorbic acid was used as the positive control. DPPH scavenging capacity was calculated by using the following formula:

% inhibition = (Abs control-Abs test sample / Abs control) * 100

Abs=Absorbance.

Table 7: % scavenging activity

S. no	Sample	HyptisSuveolens	% Scavenging activity	Tribulus Terrestris	% Scavenging activity
1	Water	50.4 ppm	49.38 %	51.8 ppm	47.93 %
2	N-hexane	55.1 ppm	44.62 %	57.7 ppm	42.01 %
3	Methanol	55.6 ppm	45.32 %	55.6 ppm	44.12 %
4	Chloroform	61.8 ppm	37.88 %	62.5 ppm	37.18 %
5	Control (DMSO)	99.5 ppm	54.67 %	99.5 ppm	54.67 %
6	Standard (Ascorbic acid)	98.8 ppm	0.70 %	98.8 ppm	0.70 %

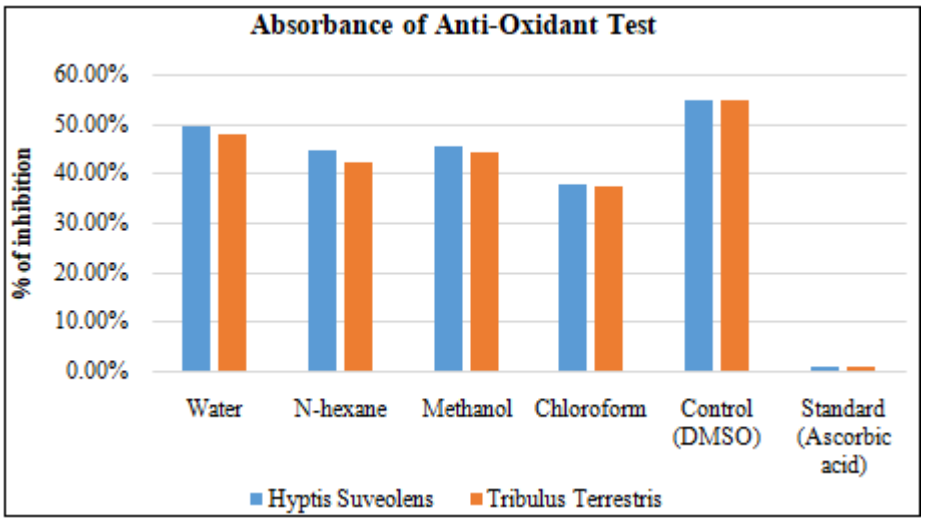


Figure 3: % Scavenging activity

Gelatinzymography Test:

Amongst all the seven compounds tribulusterrestris (N-hexane) has shown execellentanti inflammatory activity against matrix metalloproteinase-2 (MMP-2) with percentage inhibition of 98 and very good anti inflammatory activity against matrix metalloproteinase-9 (MMP-9) with percentage inhibition of 80.

Table 8

S. no	Name of the compound	%bands of MMP (MMP2)	%bands of MMP (MMP9)	%inhibition of MMP (MMP2)	%inhibition of MMP (MMP9)
1	hyptissuveolens (chloroform)	22 %	35 %	78 %	65 %
2	tribulusterrestris (N-hexane)	02 %	20 %	98 %	80 %
3	tribulusterrestris (chloroform)	12 %	26 %	88 %	74 %
4	tribulusterrestris (methanol)	20 %	25 %	80 %	75 %
5	Negative control	100 %	100 %	0 %	0 %
6	Positive control	0 %	12 %	100 %	88 %

In-Vivo Studies:

Carrageenen Induced Model:

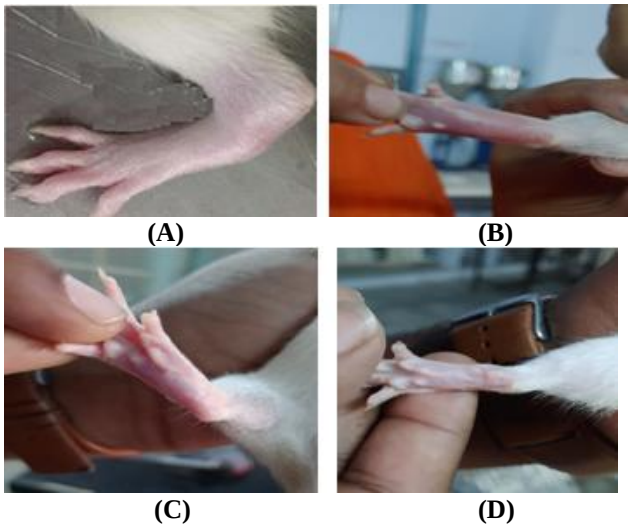
After injecting carrageenen, rats were given the test dose of both samples of hyptissuoveolens and tribulusterrestris were given for a certain period before testing of individual animal groups and standard samples is diclofenac sodiumon the day of testing, and one set of a control group that remains silent, after inducing a particular amount of drug, then the animals begin to show some changes where the sample was injected then the time and sizing were noticed for every 30min, 1hr, 2hr, 3hr, and 24hrs. The values were observed by the Vernier caliper.

Table 9: Effect of chloroform extract of Hyptis Suoveolens (seeds) on Carrageenen induced paw edema in rats

S. no	Body weight	Treatment	Average paw volume							
			0 min		30 min		60 min		120 min	
			R	L	R	L	R	L	R	L
1.	160 gm	Normal	0.6 cm	0.6 cm	-	-	-	-	-	-
2.	155.68 gm	Control	0.7 cm	0.7 cm	0.7 cm	1.04 cm	1.02 cm	1.01 cm	1.10 cm	0.8 cm
3.	170.15 gm	Standard (10mg/kg)	0.7 cm	0.7 cm	0.7 cm	1.04 cm	1.13 cm	1.01 cm	1.25 cm	0.8 cm
4.	155 gm	Test drug (100mg/kg)	0.8 cm	0.8 cm	0.8 cm	1.09 cm	1.07 cm	1.15 cm	1.15 cm	1.02 cm
5.	164.56 gm	Test drug (150mg/kg)	0.8 cm	0.8 cm	1.05 cm	1.09 cm	1.17 cm	1.15 cm	1.25 cm	1.02 cm
6.	168.65 gm	Test drug (200mg/kg)	0.75 cm	0.7 cm	0.8 cm	1.09 cm	1.23 cm	1.15 cm	1.28 cm	1.02 cm

Table 10: Effect of N-hexane extract of TribulusTerrestris (fruit) on Carrageenen induced paw edema in rats:

S. no	Body weight	Treatment	Average paw volume							
			0 min		30 min		60 min		120 min	
			R	L	R	L	R	L	R	L
1.	158.36 gm	Normal	0.6 cm	0.6 cm	-	-	-	-	-	-
2.	160.68 gm	Control	0.7 cm	0.7 cm	0.7 cm	1.04 cm	1.02 cm	1.01 cm	1.10 cm	0.8 cm
3.	168.15 gm	Standard (10mg/kg)	0.7 cm	0.7 cm	0.7 cm	1.04 cm	1.13 cm	1.01 cm	1.25 cm	0.8 cm
4.	175.28 gm	Test drug (100mg/kg)	0.8 cm	0.8 cm	1.14 cm	1.09 cm	1.22 cm	1.15 cm	1.30 cm	1.02 cm
5.	165.10 gm	Test drug (150mg/kg)	0.7 cm	0.7 cm	1.05 cm	1.05 cm	1.24 cm	1.13 cm	1.35 cm	1.07 cm
6.	159.86 gm	Test drug (200mg/kg)	0.8 cm	0.7 cm	1.10 cm	1.05 cm	1.28 cm	1.13 cm	1.34 cm	1.07 cm



(A) **(B)**

(C) **(D)**

Figure 4: Carrageenen induced

A. Before inducing at (0 min)
B. After inducing (at 120 min)
C. After inducing (at 360 min)
D. After 24 hours of inducing

Egg Albumin Induced Inflammation

After injecting egg albumin, rats were given the standard sample of diclofenac sodiumon the day of testing and a test dose of both samples of hyptissuoveolens and tribulusterrestris were given for a certain period before testing of individual animal groups and one set of a control group that remains silent, after inducing a particular amount of drug, then the animals begin to show some changes where the sample was injected then the time and sizing were noticed for every 30min, 1hr, 2hr, 3hr, and 24hrs. The values were observed by the Vernier caliper.

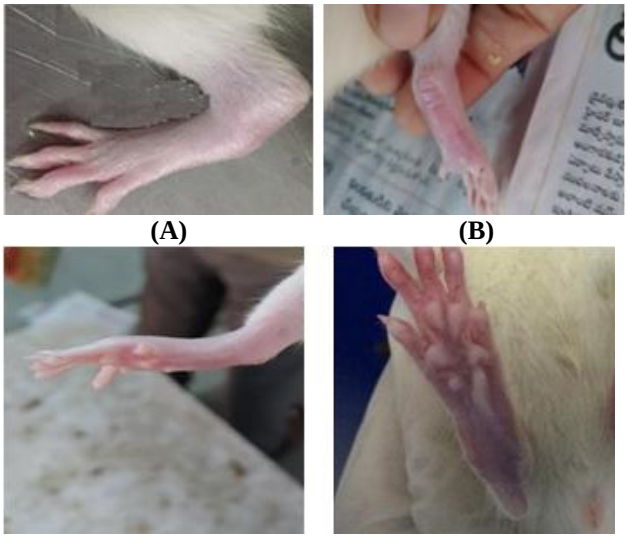
Table 11: Effect of chloroform extract of HyptisSuoveolens (seeds) on Egg albumin induced inflammation

S. no	Body weight	Treatment	Average paw volume							
			0 min		30 min		60 min		120 min	
			R	L	R	L	R	L	R	L
1.	165.10 gm	Normal	0.6 cm	0.6 cm	-	-	-	-	-	-
2.	175.28 gm	Control	0.8 cm	0.7 cm	1.15 cm	1.04 cm	1.35 cm	1.01 cm	1.60 cm	0.8 cm
3.	168.25 gm	Standard (10mg/kg)	0.8 cm	0.7 cm	1.09 cm	1.04 cm	1.27 cm	1.01 cm	1.40 cm	0.8 cm

4.	160.68 gm	Test drug (100mg/kg)	1 cm	0.8 cm	1.28 cm	1.09 cm	1.46 cm	1.15 cm	1.75 cm	1.02 cm
5.	158.56 gm	Test drug (150mg/kg)	1cm	0.8 cm	1.18 cm	1.09 cm	1.38 cm	1.15 cm	1.50 cm	1.02 cm
6.	162.74 gm	Test drug (200mg/kg)	0.95 cm	0.8 cm	1.19 cm	1.05 cm	1.25 cm	1.13 cm	1.30 cm	1.07 cm

Table 12: Effect of N-hexane extract of Tribulus Terrestris (fruit) on Egg albumin induced inflammation

S. no	Body weight	Treatment	Average paw volume							
			0 min		30 min		60 min		120 min	
			R	L	R	L	R	L	R	L
1.	164.56 gm	Normal	0.6 cm	0.6 cm	-	-	-	-	-	-
2.	155 gm	Control	0.8 cm	0.7 cm	1.15 cm	1.04 cm	1.35 cm	1.01 cm	1.60 cm	0.8 cm
3.	170.15 gm	Standard (10mg/kg)	0.8 cm	0.7 cm	1.09 cm	1.04 cm	1.27 cm	1.01 cm	1.40 cm	0.8 cm
4.	155.28 gm	Test drug (100mg/kg)	0.7 cm	0.8 cm	1.25 cm	1.09 cm	1.45 cm	1.15 cm	1.70 cm	1.02 cm
5.	160.10 gm	Test drug (150mg/kg)	0.7 cm	0.7 cm	1.18 cm	1.05 cm	1.38 cm	1.13 cm	1.55 cm	1.07 cm
6.	166.52 gm	Test drug (200mg/kg)	0.8 cm	0.7 cm	1.15 cm	1.05 cm	1.22 cm	1.13 cm	1.28 cm	1.07 cm



(A) **(B)** **(C)** **(D)**

Figure 5: Egg albumin induced

A. Before inducing (0min)
B. After inducing (120 min)
C. After inducing (360 min)
D. After 24 hours of inducing

Hot-Plate Model:

In this hot plate method, rats were given the standard sample of diclofenac sodium on the day of testing and a test dose of both samples of hyptissuoveolens and tribulusterrestris were given for a certain period before testing of individual animal groups and one set of a control group that remains silent, after placing the rats on a hot plate for about 6 to 8 sec or 15 sec, the rat was placed out of the plate then the animal begins to show some changes where the damage was placed then the time and sizing were noticed for every 30min, 1hr, 2hr, 3hr, and 24hrs. The values were observed by the Vernier caliper.

Table 13: Effect of chloroform extract of HyptisSuoveolens (seeds) on Hot-plate method

S. no	Body weight	Treatment	Average paw volume							
			0 min		30 min		60 min		120 min	
			R	L	R	L	R	L	R	L
1.	158.36 gm	Normal	0.6 cm	0.6 cm	-	-	-	-	-	-
2.	160.68 gm	Control	0.7 cm	0.7 cm	0.7 cm	0.7 cm	1.02 cm	1.02 cm	1.10 cm	1.10 cm
3.	168.15 gm	Standard (10mg/kg)	0.8 cm	0.8 cm	0.8 cm	0.8 cm	1.04 cm	1.04 cm	1.10 cm	1.10 cm
4.	175.28 gm	Test drug (100mg/kg)	0.6 cm	0.6 cm	0.6 cm	0.7 cm	0.8 cm	0.8 cm	1 cm	1 cm
5.	165.10 gm	Test drug (150mg/kg)	0.7 cm	0.7 cm	1.05 cm	1.05 cm	1.14 cm	1.14 cm	1.27 cm	1.27 cm
6.	156.38 gm	Test drug (200mg/kg)	0.7 cm	0.7 cm	1 cm	1 cm	1.11 cm	1.11 cm	1.19 cm	1.19 cm

Table 14: Effect of N-hexane extract of Tribulus Terrestris (fruit) on Hot-plate method

S. no	Body weight	Treatment	Average paw volume							
			0 min		30 min		60 min		120 min	
			R	L	R	L	R	L	R	L
1.	170.15 gm	Normal	0.6 cm	0.6 cm	-	-	-	-	-	-
2.	155.28 gm	Control	0.7 cm	0.7 cm	0.7 cm	0.7 cm	1.02 cm	1.02 cm	1.10 cm	1.10 cm
3.	160.10 gm	Standard (10mg/kg)	0.8 cm	0.8 cm	0.8 cm	0.8 cm	1.04 cm	1.04 cm	1.10 cm	1.10 cm
4.	155 gm	Test drug (100mg/kg)	0.8 cm	0.8 cm	1 cm	1 cm	1.07 cm	1.07 cm	1.15 cm	1.15 cm
5.	164.56 gm	Test drug (150mg/kg)	0.8 cm	0.8 cm	1.11 cm	1.11 cm	1.27 cm	1.27 cm	1.35 cm	1.35 cm
6.	169.25 gm	Test drug (200mg/kg)	0.8 cm	0.8 cm	1.08 cm	1.08 cm	1.15 cm	1.15 cm	1.28 cm	1.28 cm

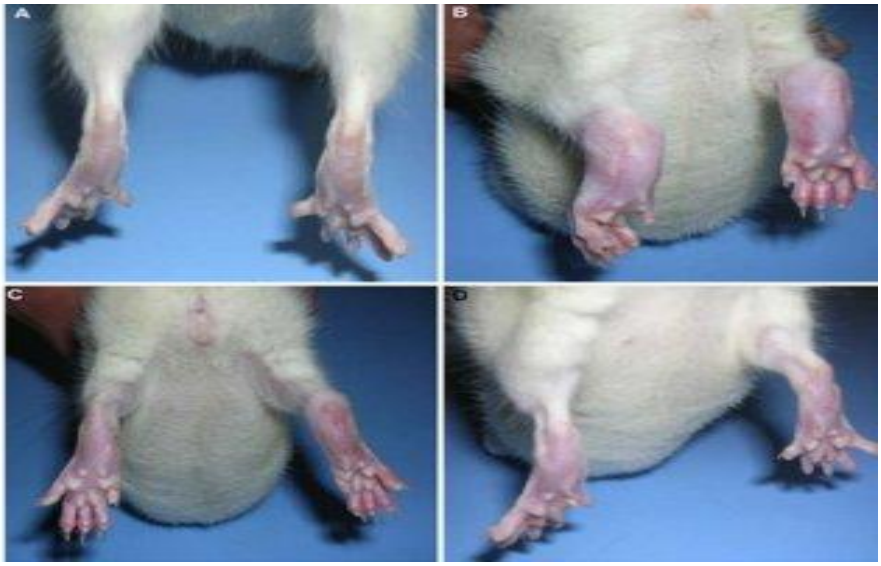


Figure 6: Hot plate model

A: Before Inducing at 0 mins
B: After Inducing at 120 mins
C: After Inducing at 360 mins
D: After inducing at 24 hours

4. Conclusion

The findings of the present study have demonstrated that HyptisSuoveolens and TribulusTerrestris both shows the potent anti-inflammatory and anti-oxidant activity and justify its use in traditional medicine to treat inflammation and painful conditions. The results also furnish evidence that the beneficial effects of this plant may be due to its free radical scavenging activity.

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