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Antiarthritic and Thrombolytic Activities of Leaves Extract of Terminalia Catappa

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ABSTRACT: The modern have a look at an ethanolic extract of Terminalia catappa's anti-arthritic and thrombolytic activity in vitro. Phytochemical evaluation of an ethanolic extract of Terminalia catappa revealed the presence of several physiologically energetic phytochemicals inclusive of phenols, flavonoids, triterpenoids, steroids, alkaloids, and others. The fruit can assist with bronchitis and bowel problems. The juice of the leaves is used to make an ointment for scabies, leprosy, and cutaneous illnesses, in addition to an analgesic. Dysentery and diarrhoea are treated with the basic bark. The stem bark is used to deal with fevers. numerous pharmacological research have established that this plant has antimicrobial, anti-inflammatory, antidiabetic, antioxidant, hepatoprotective, and anticancer properties, all of which assist its conventional uses. due to the fact that these compounds are of pharmacological interest, and because this plant is used in traditional medicinal drugs, we decided to check all terminalia catappa plant leaves for in vitro antiarthritic activity using the protein denaturation approach and thrombolytic activity using human blood. The ethanolic extracts of Terminalia catappa leaves have been determined to have anti-arthritic and thrombolytic residences. At a dose of 1000 µg/ml, the inhibition of protein denaturation was discovered to be 78.54 ± 2.05 percent, and the percent clot lysis was located to be 42.32 ± 1.38 percent in thrombolytic hobby. As a result, our findings guide the isolation and use of active elements from Terminalia catappa leaves in vitro for arthritis treatment and clot lysis.

Keywords Phytoconstituents, Terminalia catappa, Antiarthritic and thrombolytic activity.

I. INTRODUCTION

Globally around 80,000 plant species are used for medicinal and aromatic purposes. Despite the widespread use of herbal medicine, rapid urbanization, migration, climate change, and the growing number of modern healthcare systems around the world have influenced traditional knowledge of medicinal plant use [1-4]. About 90% of the herbal raw drugs used in the manufacture of vegetable drugs come from a limited wild source. The use of medicinal plants is expected to increase globally as herbal medicine and Ayurveda gain in popularity. The increased use of herbs is required due to the increased adverse effects and cost of synthetic drugs [5]. Rheumatoid Arthritis (RA) is an autoimmune disease that affects about 5% of the human population [6]. RA is a type of chronic inflammatory polyarthritis that affects multiple diarthrodial joints in a specific pattern, causing pain, deformities, and a lower quality of life. RA is characterized by extensive synovitis, which causes articular cartilage and marginal bone erosion, resulting in joint destruction [7]. The metacarpophalangeal, proximal interphalangeal, wrist, and metatarsophalangeal joints are the most common sites of onset synovitis, though any joint can be affected. Fatigue, malaise, weight loss, fever, and depression are some of the constitutional symptoms that can occur prior to the onset of RA [8]. Thrombosis is the formation or presence of a blood clot in a blood vessel. Venous thrombosis and Arterial thrombosis are the 2 most not unusual types of thrombosis, based totally

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definitely totally on wherein the clot paperwork. To dissolve clots, thrombolytic remedy uses tablets called thrombolytic sellers, which incorporate alteplase, anistreplase, streptokinase, urokinase, and tissue plasminogen activator (TPA). Blood clots that form in catheters or tubes inserted into human beings's bodies for clinical treatments like dialysis or chemotherapy are also treated with thrombolytic treatment. However, due to the reality first-technology outlets (streptokinase and urokinase) have a low substrate specificity, they'll be capable of causing systemic fibrinolysis and bleeding complications. due to the rules of currently to be had thrombolytic drug, efforts are being made to boom advanced recombinant versions [9-13]. Terminalia catappa Linn. It is a tropical ornamental tree of the Combretaceae family, native to Southeast Asia, Africa and Australia. Bengal Almond, Wild Almond, False Kamani, Indian Almond, Malabar Almond are some common names for this tree. It is 35 meters high, has a symmetrical canopy [14] and horizontal branches, extending to 9 meters. Discrete light yellow-green leaflets are grouped in axillary ears. Flowering usually begins 2-3 years after planting, but this will vary depending on location and genotype. It is a large and solemn deciduous tree with a characteristic pagoda shape. [15]. It is cultivated in Nigeria exclusively as a shade tree and for its fruits and seeds as well as for medicinal purposes [16]. Terminalia catappa has been identified, characterized and standardized as a plant of medicinal importance in several reports [17,18]. Different parts of this plant are of medicinal importance and exhibit a variety of activities: antibacterial activity [18], antioxidant and hepatoprotective activity [19], anti-inflammatory activity [20], antifungal, anticandidal and antioxidant activity [21] in the leaves of Terminalia catappa shown.

II. MATERIALS AND METHODS

Sample collection and preparation

The fresh leaves of Terminalia catappa Linn. It was collected by the Sahyadri College of Pharmacy, Methwade, Sangola, Solapur. The taxonomic identification of the plant was carried out by Dr. Tembhurne R.R, Department of Botany, Sangola college, Sangola, using the flora of the Solapur District, Maharashtra, India. The specimens were deposited in the Herbarium, Department of Botany, Sangola College, Sangola. leaves were dried in the shade, coarsely pulverized and stored in an airtight container for later use.

Preparation of extracts

The extracts were produced using a Soxhlet apparatus, various extracts such as the chloroform extract, the ethyl acetate extract and the ethanol extract being produced using these solvents, the extraction was carried out in a Soxhlet extractor for 72 hours The extracts were hot with Whatman filter paper (No. 1) filtered, concentrated in vacuum under reduced pressure using a rotary flask evaporator and dried in desiccators. Preparation of aqueous extracts 250 g coarse The powder is macerated with 500 ml of water for 24 hours, chloroform is added to prevent bacterial growth, and then the extract is concentrated for later use.

Phytochemical test:

A) Detection of alkaloids

1) Wagner test: A few drops of Wagner's reagent (iodine in potassium iodide) were added to the extract. The presence of alkaloids is indicated by the formation of a red-brown precipitate.

2) Mayer's test: some drops of Mayer's reagent (potassium mercury iodide) were added in extract. The presence of alkaloids is indicated by the formation of a cream colored precipitate.

3) Dragendorff test: a few drops of Dragendorff reagent (solution of potassium bismuth iodide) The formation of a red-brown precipitate indicates the presence of alkaloids.

B) Detection of glycosides

1) Legal test: To extract, add 1 ml of pyridine and a mixture of sodium nitroprusside and sodium hydroxide. indicates the presence of glycosides.

2) Keller-Killiani test: 2 ml of extract mixed with glacial acetic acid, 5 drops of FeCl3 and concentrated H2SO4. A red-brown color appears at the junction of the two layers of liquid, while the top layer appears green and bluish. .

C) Flavonoid detection

1) Alkaline test: A few drops of sodium hydroxide solution were added to the extract. The presence of an intense yellow color, which fades to colorless with the addition of dilute acid, indicates the presence of flavonoid.

2) Lead acetate test: A few drops of lead acetate solution were added to the extract. The presence of flavonoids was indicated by the formation of a yellow precipitate.

D) Detection of saponins

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1) Foam test: Shake the drug extract vigorously with water. Persistent foam is observed.
E) Detection of sterols

1) Salkowski test: Add 2 ml of extract, 2ml of Chloroform and 2 ml Of concentrated H2SO4 and shake well. The chloroform layer appears red and the acid layer shows greenish-yellow fluorescence.
2) Liebermann-Burchard test: 2 ml of extract + 2 ml of chloroform. From the side of the test tube add 1-2 ml of acetic anhydride and 2 drops of concentrated H2SO4. First red appears, then blue and finally green[22-24].

a) Arthritic Activity

In the current study, the anti-arthritic activity of Terminalia catappa is assessed in vitro models. The standard is diclofenac sodium.

Preparation of reagents:

1 % Bovine serum albumin:

Dissolve 1 gm of BSA in 100 ml Distilled water.

Preparation of standard solution:

Diclofenac sodium is used as the standard. A stock solution of diclofenac sodium in water of 1000 µg/ml was made. From this stock solution, 3 different concentrations of 50, 100, 500 and 1000 µg/ml were obtained.

Preparation of Test solution:

Stock solutions of the various root extracts of 1000 µg/ml were prepared using ethanol, ethyl acetate, chloroform and water as solvents, from this stock solution 3 different concentrations of 50, 100, 500 and 1000 µg/ml were made.

In vitro Anti-arthritic activity

This activity was evaluated by the albumin denaturation test, plant extracts were prepared in concentrations of 50 to 1000 µg/ml, a reaction mixture was prepared for each concentration with 1 ml of test drug and 1 ml of 1% bovine albumin solution. These prepared solutions were incubated for 15 minutes at 27 ° C. The reaction mixtures were kept in a water bath at 70 ° C. for 10 minutes to induce denaturation. The solutions were cooled before being measured for turbidity spectrophotometrically at 660 nm. Diclofenac sodium was used as a standard drug at concentrations ranging from 50 to 1000 µg / ml and the test extracts were treated similarly. The percent inhibition of denaturation was calculated using a control group in which no drug was added. Each experiment was repeated three times using equation [25] to calculate the percent inhibition of protein denaturation.

% Inhibition of protein denaturation = 100 x [A 1 - A 2 / A1]

where, A 1 = control absorption

A 2 = test sample / standard absorption in albumin solution

b) Thrombolytic Activity:

For this study Aspirin is used as Standard.

Preparation of reagents:

Preparation of standard solution:

Aspirin is used as standard. Stock solution of Aspirin in water was prepared of 1000 µg/ml. From this stock solution 5 different concentrations of 200, 400, 600, 800 and 1000 µg/ml. were prepared.

Preparation of Test solution:

Stock solutions of the various leaves extract of 1000 µg/ml. were prepared by using ethanol, ethyl acetate, chloroform and water as solvent. From this stock solution 5 different concentrations of 200, 400, 600, 800 and 1000 µg/ml. were prepared.

Thrombolytic Activity:

Venous blood samples (3 ml each) were taken from three healthy volunteers. 500 µl of blood was transferred in vitro to each of the five Eppendorf tubes previously weighed for each subject. 45 minutes at 37 ° C. After clot formation, all of the serum was withdrawn and each tube containing the clot was reweighed to determine the weight of the clot (weight of clot = weight of tube containing the clot - the weight of the tube alone). The clot was weighed, 200-1000 µg/ml of various concentrations of plant extracts or 100 µl L distilled water was added as a negative control, then all tubes were incubated for 90 minutes at 37 ° C to verify clot lysis. After incubation, the release of liquid was stopped and the tubes were reweighed to determine if there was a weight difference after the clot stabilized. The weight difference obtained was expressed as the percentage of stable clot or lysis. Experimental series, simultaneous addition of 500 µl blood and 100 µl aspirin, incubated at 37 ° C for 45 minutes. The weight of the clot obtained was determined as indicated above [26].

The percentage (%) of clot lysis was measured as,

% of clot lysis = (weight of the released clot / weight of the clot) × 100

= (W2 - W3 / W2 - W1) × 100

Where,

W1 = empty weight of the Eppendorf tube.

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W2 = weight of the Eppendorf Tube + clot Weight.
W3 = weight of the clot released after adding the plant extract

Statistical analysis

ANOVA was used to analyze the data followed by a Student T test using Graph Pad Prism Data Editor for Windows. The mean and standard error of the mean (SEM) were used to express the data. The statistical significance was defined as P< 0.05-0.01.

III. RESULT AND DISCUSSION

Qualitative analysis:

The results of the qualitative phytochemical test of terminalia catappa leaves extract of different extracts are given in the below table. 1.

Table 1: Result of Qualitative Phytochemical Test of Terminalia Catappa Leaves Extract

Sr No.	Chemical constituent	Chemical test	Ethanol	Ethyl acetate	Chloroform	Aqueous
1.	Alkaloid	Mayer's test	+	-	+	+
		Hager's test	+	+	+	-
		Dragendorff's test	+	+	+	+
2.	Glycosides	Keller-Killiani test	+	+	+	+
		Legal's test	-	+	-	-
3.	Flavonoids	Alkaline test	+	+	+	-
		Lead acetate test	+	-	+	+
4.	Saponin	Foam test	+	-	-	+
5.	Sterol	Salkowski test	+	+	+	-
		Libermann-Burchard test	+	+	-	+

(+ve sign denoted as test is present and –ve sign denoted as the test is absent)

In vitro anti arthritic activity

The extracts were found to be effective as anti-arthritic agents and showed significant activity compared to the standard drug. Anti-arthritic activity was also shown in a concentration-dependent manner. The activity was reported at the highest concentration used for evaluation. The ethanol extract was found to be more effective than the other extracts, showing 78.54 ± 2.05% inhibition of denaturation of the protein, while 66.95 ± 1.56%, 64.05 ± 1.75% and 60.86 ± 1.56% was achieved by ethyl acetate, chloroform and aqueous extract at a concentration of 1000 µg/ml, respectively which was the highest concentration evaluated. The percentage of inhibition by the extracts at various concentrations and their comparison with the standard drug are shown in Table 2 and in figure 1. The IC 50 values for ethyl acetate, ethanol, chloroform and aqueous extracts were 495.89, 87.27, 731.42 and 685 µg/ml, respectively which further confirmed that the ethanol extract was more effective.

Table 2. % Inhibition of Protein Denaturation of Different Leaves Extracts of Terminalia Catappa by In-Vitro Method

Sr. No.	Plant Extract	Concentration (µg/ml)	% Inhibition ± SEM
1.	Standard (Aspirin)	50	52.17 ± 1.15
		100	64.63 ± 2.23
		500	75.07 ± 1.39
		1000	87.24 ± 1.32
2.	Ethanol	50	40.28 ± 1.32
		100	57.67 ± 1.52
		500	66.95 ± 1.56

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		1000	78.54 ± 2.05
3.	Ethyl acetate	50	19.99 ± 1.99
		100	33.33 ± 2.18
		500	57.67 ± 1.52
		1000	66.95 ± 1.56
4.	Chloroform	50	14.20 ± 1.32
		100	22.60 ± 1.56
		500	37.10 ± 1.52
		1000	64.05 ± 1.75
5.	Aqueous	50	16.81 ± 1.09
		100	25.79 ± 1.32
		500	49.56 ± 1.15
		1000	60.86 ± 1.56

(Each Value are expressed as Mean ± SEM of 3 reading)

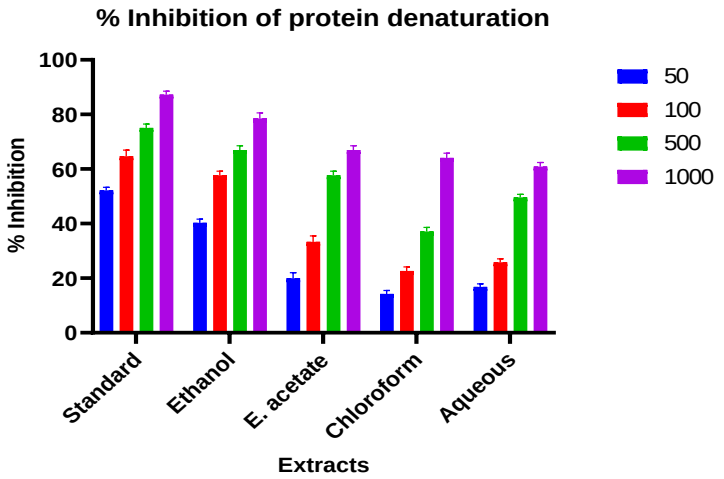

Figure 1. Antiarthritic activity of different extracts of Terminalia catappa leaves

In vitro thrombolytic activity

As part of the discovery of thrombolytic agents from natural extracts, Terminalia catappa leaves were examined for thrombosis. The results are shown in Table 3 and Figure 2. 1 ml of aspirin as a positive control (1000 µg / ml), which was added to (clots) and then incubated for 90 minutes at 37 ° C. In contrast, showed a clot lysis of 64.24 ± 1.71%. Distilled water was used as a negative control, which resulted in a negligible percentage of clot lysis (4.00 ± 0.03%). The terminalia catappa leaf ethanolic extract showed a higher level of thrombolytic activity at all concentrations. An increase in clot lysis activity was observed at 42.32 ± 1.38% of the ethanol extract (1000 µg / ml). The minimum of clot lysis was observed in the chloroform extract of terminalia catappa.

Table 3: % Clot Lysis of Different Leaves Extract of Terminalia Catappa by In-Vitro Method

Sr. No.	Concentration of plant extract (µg/ml)	% clot thrombolysis				Blank negative control	Aspirin positive control
		Ethanol	Ethyl acetate	Chloroform	Aqueous		
1.	200	29.66±0.48	20.10±1.77	16.75±1.21	19.20±1.48	4 ±	64.24 ±

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2.	400	31.86±0.98	22.16±1.26	18.87±1.23	21.33±2.40	0.037	1.71
3.	600	35.26±0.91	24.76±1.34	20.58±1.48	25.20±1.96		
4.	800	37.47±1.04	30.18±1.88	23.94±0.80	28.02±1.10		
5.	1000	42.32±1.38	34.96±1.63	28.93±0.84	33.35±1.28		

(Each Value are expressed as Mean ± SEM of 3 reading)

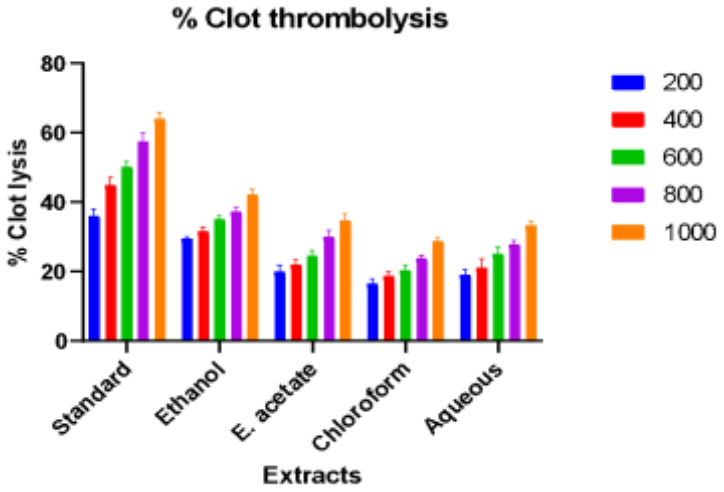

Fig 2: Thrombolytic activity of different extracts of Terminalia catappa leaves

IV. CONCLUSIONS

The ethanol extract of Terminalia catappa showed better anti arthritic activity, thrombolytic and anticancer activity than the ethyl acetate, chloroform and aqueous extracts. The future scope of study involves the isolation of phytoconstituents and mechanisms responsible for the anti arthritic activity, thrombolytic and anticancer activity.

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