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Anti-Inflammatory Activity of Selected Flowers Extract Preparation - an *In Vitro* Analysis

Surya Chinnasamy, *Archana V. G[1], Sujithra.M[2], P. Shobana Devi[3] and Deepika Singh[4]
*Department of Biochemistry Dhanalakshmi Srinivasan college of Arts and science for women (Autonomous) Perambalur 621212, Tamilnadu, India
[1,2,3,]Department of Biochemistry,Dhanalakshmi Srinivasan college of Arts and science for women (Autonomous) Perambalur 621212, Tamilnadu, India
[4]Sr. Asst. Prof. Babu Banarasi Das University, Lucknow, U.P., INDIA

ABSTRACT
Anti-inflammatory agents block certain substances in the body that cause inflammation. They are used to treat many different conditions. Some anti-inflammatory agents are being studied in the prevention and treatment of cancer. The therapeutics position of quantity of Neem flowers in ailments administration is nonetheless being enthusiastically researched due to their much less aspect impact and low-priced properties. It has been popular that capsules primarily based on allopathy are steeply-priced and additionally showcase poisonous impact on everyday tissues and on a range of organic activities. The Moringa genus has historically been broadly used to enhance health. Kings and queens used Moringa to enhance their alertness and to keep healthful skin. Indian warriors have been fed M. oleifera leaves to beautify their electricity and assist to relieve their ache and stress all through combat. It is a mostly popular truth that severa pharmacologically energetic capsules are derived from herbal assets consisting of medicinal flowers The flower Moringa oleifera belongs to family Moringaceae and Azadirachta indica belongs to family Maleacea and Moringa oleifera belongs to family Moringaceae and Crepe jasmine belongs to family Apocynaceae. The flowers of Azadirachta indica, Moringa oleifera, Tanner's cassia and Crepe jasmine were collected and thoroughly dried under shade and powdered mechanically, phytochemical analysis of the plant drug was carried out was carried out, Anti-inflammatory exercise of flower extract was once evaluated towards denaturation of egg albumin technique, HRBC suspension was used for the estimation of anti-inflammatory property. ADMET test was carried out by SWISS ADME. The result revealed the Flower extract and has significant Anti-inflammatory activity.
Keywords: *Moringaceae, Azadirachta indica, Apocynaceae, phytochemicals, anti-inflammatory, ADMET test, SWISS ADME*

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INTRODUCTION

Tanners Senna (Family: Caesalpiniaceae) holds a very prestigious role in Ayurveda and Siddha structures of medicine. Its bark is used as an astringent, leaves and fruits are used anthelminthic [11]. An alternative guidance for diabetes medicinal drug is a combination referred to as "avarai panchaga choornam" which is organized from dried and powdered plant components and generally used for opthalmia, con-junctivitis, and urinary infections (equal quantity of leaves, roots, flowers, bark, and unripe fruits) [4]. The plant has been pronounced to possess antipyretic [8], hepatoprotective[2], anti-peroxidative[1] and microbicidal recreation. The therapeutics position of quantity of Neem flowers in ailments administration is nonetheless being enthusiastically researched due to their much less aspect impact and low-priced properties. It has been popular that capsules primarily based on allopathy are steeply-priced and additionally showcase poisonous impact on everyday tissues and on a range of organic activities. It is a mostly popular truth that several pharmacologically energetic capsules are derived from herbal assets consisting of medicinal flowers [13] The Moringa genus has historically been broadly used to enhance health. Kings and queens used Moringa to enhance their alertness and to keep healthful skin. Indian warriors have been fed M. oleifera leaves to beautify their electricity and assist to relieve their ache and stress all through combat [7].

Currently, it is every day that the plant has anti-inflammatory, antioxidant, anticancer, and antidiabetic activities. Recently, extra lookup has been carried out on different species such as *M. concanensis, M. stenopetala,* and *M. peregrina*. The genus, *Tabernaemontana* contains of a hundred species with a large -7-distribution in the tropical and subtropical components of Asia, Africa, Americas, Oceania and Australia. In fact, about thirteen special species are discovered in Malaysia [6]. Documented phytochemical research

have proven *Tabernaemontana* as a supply of novel structured indole and bisindole alkaloids possessing fascinating bioactivities with the most surprisingly anticancer property [5].

There are a variety of drugs for controlling and suppressing inflammatory crisis; steroids, nonsteroid anti-inflammatory drugs, and immunosuppressant are the sensible examples of these medicines which are related with negative results whilst in exercise our intention is to observe minimal wonderful dose by means of the best possible efficacy with the least a diverse effect. Thus, we want to observe herbal anti-inflammatory elements inside medicine remedy to obtain elevated pharmacological response and the lowest diploma of undesirable facet consequences [3].

Sample Collection

The flowers of *Moringa oleifera*, Tanner's cassia, *Azadirachta indica*, Crepe jasmine collected, thoroughly dried under shade and powdered mechanically and sieved through No.20 mesh sieve. The finely powdered leaves and flowers were kept in an airtight container until the time of use.Powdered bark (20 g) was extracted in 200 mL of 95% ethanol for 4-5 h in a Soxhlet extractor (4–6 cycles) until the solvent in the siphon tube become colourless. The extract was filtered and evaporated to dryness under vacuum in a rotary evaporator.

Qualitative phytochemical analysis

Test for Tannins: 10 ml of bromine water was added to the 0.5 g aqueous extract. Decoloration of bromine water showed the presence of tannins.

Test for Saponins: 5.0 ml of distilled water was mixed with aqueous crude plant extract in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins.

Tests for Flavonoids: Alkaline Reagent Test. 2 ml of 2.0% NaOH mixture was mixed with aqueous plant crude extract; concentrated yellow color was produced, which became colorless when added 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.

Detection of Phenols: Ferric chloride test: extracts were treated with few drops of 10% ferric chloride solution. Formation of bluish black colour indicates that the presence of phenol.

Test for Steroids: 2 ml of chloroform and concentrated H2SO4 were added with the 5 ml aqueous plant crude extract. In the lower chloroform layer red color appeared that indicated the presence of steroids.

Test for Quinones: About 0.5 g of plant extract was taken and added 1 ml of extract and 1 ml of con.· H2SO4was added formation of red colour shows the presence of quinones -26-

Test for Carboxylic acid: One ml of the various extracts was separately treated with a few ml of sodium bicarbonate solution. Effervescence (due to liberation of carbon dioxide) indicates the presence of carboxylic acid.

Detection of Alkaloids Mayer s test: Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids. Filtrates were treated with Mayer s reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Test for Coumarins: 0.5 mL of the moistened extracts was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins

Tests for Lignins Labat test: When gallic acid is added to the test sample,it results in the formation of olive green colour indicate positive

TLC: Thin Layer Chromatography has been used as an analytical tool, especially in organic chemistry because of its high speed of separation and its applicability in a large number of chemical compounds. The high sensitivity of TLC is used to check the purity of the samples. With the help of TLC, it is possible to know whether a reaction is complete and had followed the expected course. The homogeneity of the compounds was monitored by this TLC plates and visualized by iodine vapour.

Separation: 10 µL sample is applied on pre prepared TLC plates (MREK) activated at 100° C for 5 min and then placed on a beaker contain mobile phase. A glass chromatographic tank saturated with the mobile phase for 30 min was used for linear ascending development. The developed plates were air dried and then UV scanned at 275 nm.

Inhibition of protein denaturation

Reaction mixtures were incubated in a water bath at 37°C ± 2°C for 15–20 min, and later, it was heated at 70°C at which the reaction mixture was maintained for 5 min. Then, the reaction mixture was allowed to cool down at room temperature for 15 min. Absorbance of reaction mixture before and after denaturation was measured for each concentration (100-500 mg/ml) at 680 nm using a colorimeter. Each test was repeated thrice and the mean absorbance was recorded. The percentage of inhibition of protein was determined on a percentage basis with respect to control using the following formula.

100 x (Test/control) -1

***In vitro* Anti-inflammatory activity**

HRBC method was used for the estimation of anti-inflammatory activity in vitro. Blood was collected from healthy volunteers and was mixed with equal volume of sterilized Alsevers solution. This blood solution was centrifuged at 3 000 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 estimation of anti-inflammatory property. Different concentrations of extract, reference sample and control were separately mixed with 1mL of phosphate buffer, 2 mL of hyposaline and 0.5 mL of HRBC suspension. All the assay mixtures were incubated at 37 °C for 30 minutes and centrifuged at 3 000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in the control as 100%.

Percentage protection= 100- (OD sample/ OD control) × 100

RESULTS AND DISCUSSION

Cenna auriculata , Tanner's cassia, Azadirachta indica, Moringa oleifera gathered and dried nicely (Fig.1) samples had been blended collectively and extracted and targeted below vaccum desicator(Fig.2)The phytochemical traits of the three poly natural flower exclusive extracts of (Fig.2) investigated are summarized in Table 1. The qualitative antibacterial assay printed that of the eight special check Carboxyl team and Coumarins solely had been terrible and stays are positive. Alkaloids, Flavonoids and steroids were before detected in Cassia auriculata by means of [11]. Alkaloids, steroids, flavonoids, tannins, saponins,were observed to be principal parts from Moringa oleifera stated via [12]

Table 1 Phytochemical Analysis

TEST	RESULT
Test for flavonoids	Positive
Test for phenolic	Positive
Test for sterols	Positive
Test for Quinones	Positive
Test for Carboxyl group	Negative
Test for Tannin	Positive
Test for Saponin	Positive
Test for Coumarins	Negative

Cenna auriculata ***Moringa oleifera***

T.divaricata ***Azadirachtaindica***

Fig.1 Flowers used for polyhedral

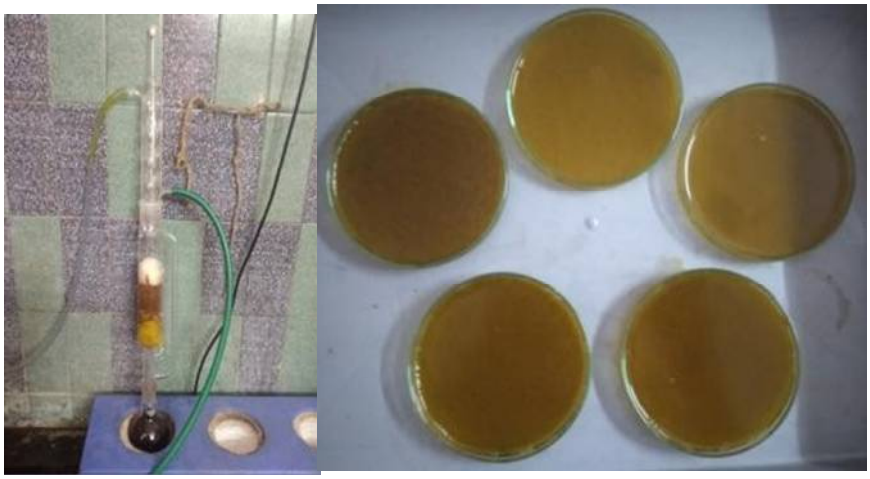

Fig. 2 Polyherbal preparation by soxhlat extraction

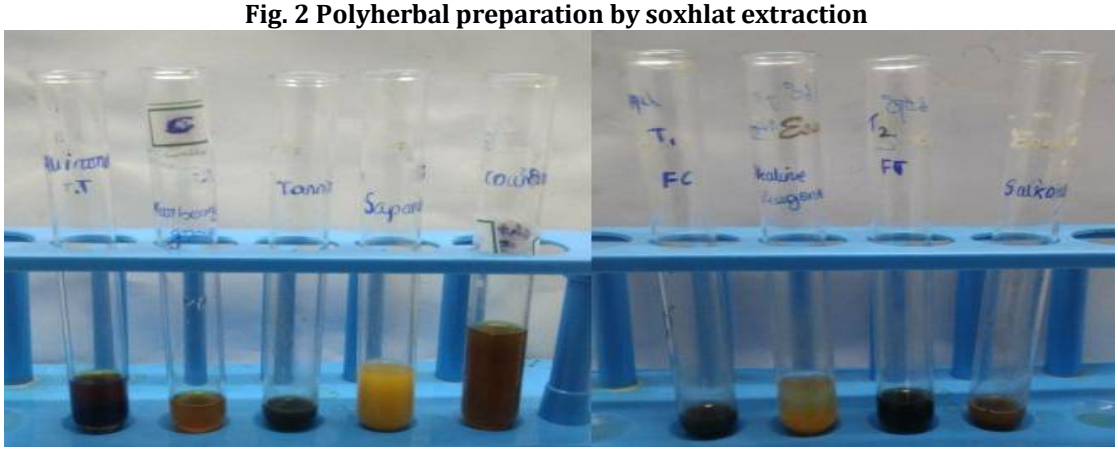

Fig.3 Phytochemical test of extract

Anti-inflammatory activity in vitro

Percentage of Anti-inflammatory pastime of HRBC and albumin denaturation is given in desk Anti-inflammatory exercise of flower extract was once evaluated towards denaturation of egg albumin technique (Fig.4). Extract carries anti-inflammatory property in opposition to egg albumin denaturation approach and has substantially greater effective recreation than reference drugs.The absolute best inhibition fee was once determined 72% for extracts at the attention of five hundred µg/ml and 65% for dichlofenac. There was once considerably greater inhibition in extractions as in contrast to popular at the concentrations of five hundred mg/ml. It was once referred to that growing awareness of extract have given growing denaturation inhibition recorded as 12% at one hundred mg/mL and expanded as 28, 42, sixty eight and 72% amongst 200,300, four hundred and five hundred mg/mL. Aqueous extracts at extraordinary concentrations (100, 200, 300, 400, five hundred mg/mL) confirmed substantial stabilization in the direction of HRBC membranes (Fig. 5). The share safety of extract at attention 300 mg/mL used to be higher (70%) than that of concentrations (36% at five hundred mg/mL). However, the proportion safety was once observed to be reduced at greater concentration. The consequences have been tabulated. HRBC technique used to be chosen for the in vitro comparison of anti-inflammatory property due to the fact the erythrocyte membrane is analogous to the lysosomal membrane [10]. Denaturation of protein has an unpredictable mechanism which consists of change in electrostatic hydrogen, hydrophobic and disulfide bonding.[9] Denaturation of protein motives the manufacturing of autoantigens in stipulations such as rheumatic arthritis, most cancers and diabetes which are stipulations of inflammation.

Table 7. Percentage of Anti inflammatory activity of polyherbal preparation

Concentration	HRBC	Albumn in denaturation
100	24	12
200	42	28
300	70	42
400	52	68
500	36	72
Dichlofenac 500	70	65

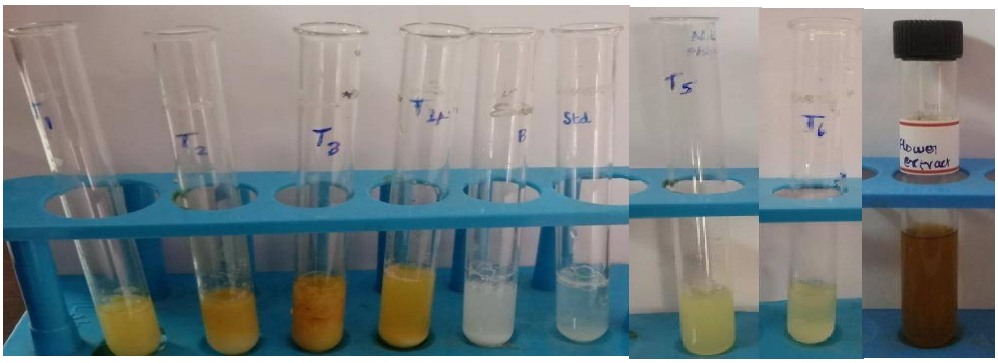

Fig.4 Anti-Inflammatory Test

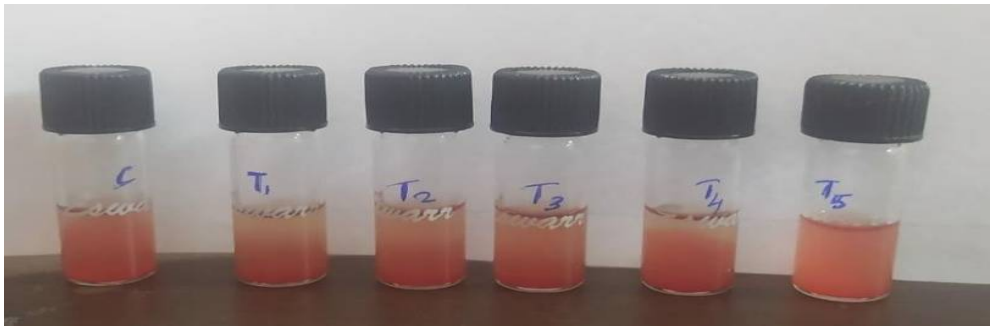

Fig.5 HRBC Test

CONCLUSION

The chemical compounds of flower extract have been recognized thru in-vitro anti-inflammatory undertaking was once assessed in human erythrocytes, egg albumin denaturation. The extract confirmed strong anti-inflammatory pastime in in-vitro assays. Affiliation of non-steroidal anti-inflammatory pills (NSAIDs) with medicinal plant extracts may also amplify its antinociceptive activity, allow the use of decrease doses and restrict facet effects. Three phytochemicals exhibited the higher aggressive end result than the traditional anti-inflammatory drug dichlofenac. The end result of HRBS and albumin denaturation inhibition denotes that the extract has anti- inflammatory property at awareness established manner. Further and certain researches are in manner for the isolation of energetic constituent accountable for this property and to identification of the feasible mechanism of its anti- inflammatory property. Both experimental and computational research have scientifically published the makes use of the experimental medicinal flower phase in inflammatory disorders.

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