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PHYTOCONSTITUENT ANALYSIS AND *IN-VITRO* ANTI-INFLAMMATORY ACTIVITIES OF PLANTS SOURCES

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ABSTRACT: Bioactive compounds are secondary plant metabolites and are found in small amounts in plants, fruit, vegetables, and edible oils; they have health benefits and provide us an alternative search for drugs or medicines and followed by the minimum side effects. There are various bioactive assays for measuring the functional activities of bioactive compounds, such as phytochemical assays and anti-inflammatory assays and so on. The phytochemical research and *in-vitro* anti-inflammatory activity research on plants is considered an effective way to discover novel bioactive compounds with potential as drug leads. Ever since ancient times, plants have been used for treating several diseases. The present research aims to assess the anti-inflammatory impacts and the phytochemical makeup of several plant extracts. In this review, we compiled information on twelve distinct plant extracts that showed the highest levels of phytochemical activity and anti-inflammatory activity, and we offer additional research directions for these bioactive components.

INTRODUCTION: Since the dawn of time, disease has been a natural part of man. Drugs are as old as sickness, and the hunt for cures is likely just as old. Longer than a thousand years, herbal medicine is widespread, ostensibly secure and efficiently, a variety of sickness symptoms to treat [1]. The best that we can tell, no thorough research on the anti-inflammatory and antibacterial qualities of various leaf extract fractions has yet been done. Consequently, an effort is made here to research the leaf extracts and their phytochemical activities and Anti-inflammatoryactivites on the twelve different plants namely *Gloriosa superba, Syzyniumcumini, Canthium parviflorum, Holarrhena antidysentria, Costus, Polyalthia longifolia, Tabernaemontana divaricata, Ficus, Tecoma stans, Tagetuserecta, Solanum Melongena, Solanum Lycopersicum*. The release of kinins, prostroglandins, and histamines by wounded tissue cells [2]. A complex process that typically results in discomfort, inflammation also increases vascular permeability, increases protein denaturation, and changes membrane properties [2]. When subjected to external stressors or substances like extreme temperatures, a strong acid or base, concentrated inorganic salts, organic solvents, or strong acids or bases, proteins can lose both their secondary and tertiary structures.

When biological proteins are denatured, most of them lose their biological function. Inflammation is known to be exacerbated by protein denaturation. An essential non-specific defense response to tissue injury, such as that induced by a disease or wound, is inflammation, which is characterized by warmth, redness, pain, and swelling [3]. Together, this results

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in enhanced capillary permeability and vasodilation (blood vessel widening). The damaged area receives more blood as a result.

Additionally, through a process known as chemotaxis, these compounds help to attract some of the body's natural defense cells using chemical messengers. Acute or chronic inflammation are two different categories. Increased blood flow of plasma and leukocytes (particularly granulocytes) into the wounded tissues sets off acute inflammation, the body's initial reaction to damaging stimuli. The biochemical series of actions that increases and develops the inflammatory reaction involves the neighborhood's vascular system, immunological system, and countless cells inside the defective tissue. The definition of chronic inflammation, also known as long-lasting inflammation, is the inflammatory process's simultaneous ability to damage and mend tissue. It results in the type of cells at the site of inflammation are changing gradually. Five *in-vitro* based assays, including the Membrane Stabilization Assay, Heat-Induced Hemolysis, Albumin Denaturation, Proteinase Inhibitory Effect, and Hypotoxicity Induced Hemolysis, were used to estimate the anti-inflammatory activity.

MATERIALS AND METHODS:

Plant Extract Preparation: Samples of leaves were gathered in and around Visakhapatnam. The leaves were fragmented and immersed in phosphate buffer solution overnight. The following day, these samples were mashed with to a fine pulp in a mortar and pestle. Later centrifuged for 20 minutes at 4000rpm. *In-vitro* anti-inflammatory tests and phytochemical analyses were performed on the obtained supernatant [4-5].

Chemicals: Phytochemical constituents, HRBC, Phosphate buffer, Bovine Albumin, Casein, Tris HCl, Trypsin, Normal saline solution.

Methods:

Phytochemical Analysis: Plant bioactive compounds play an important role in human life. Plants exhibit both primary and secondary metabolites, namely Flavonoids, Terpenoids, Phenols, Quinones, Coumarins and so on, which are useful for diagnosing various diseases. Leaf from the selected twelve plants is cut into small pieces and soaked in phosphate buffer solution for 24 hours. Soaked leaves are grounded separately with the help of a motor and pestle, and filtration is done. Centrifuge them at 4000rpm for 15 minutes. Supernatant collected is used to identify phytochemicals present in them [4].

Test for Phenols:

Lead Acetate Test: A 10% lead acetate solution is added to 1 ml of plant extract supernatant after it has been diluted with 5 ml of distilled water. When white precipitate is seen, it means that phenol is present [6].

Saponin Test:

Test of Foam: 3ml of distilled water is added to 1ml of plant extract to dilute it. Saponins are present when a 1 cm layer of foam forms [7].

Test for Tannins:

Braymer's Test: 2ml of distilled water is mixed with 2ml filtrate. Add a couple of drops of 10% $FeCl_3$ solution to this. The presence of tannins is shown by the colour change to green [8].

Test for Flavonoids:

Zinc-Hydrochloride Reduction Test: Add a few drops of petroleum ether and a few pieces of zinc dust to 1 ml of the plant sample. Afterward, dropwise add 2 ml of strong hydrochloric acid. Flavonoids are present as shown by the emergence of the magenta colour [9].

Test for Quinones:

Sulfuric Acid Test: Extract diluted in isopropyl alcohol to 1ml. 1 ml of concentrated solution to this Drop by drop, H_2SO_4 is added. Quinones are present when the colour is red [10].

Test for Alkaloids:

Bertrand's Test: Add 2 drops of potassium mercuric iodide to 1 millilitre of supernatant. Alkaloids are present when a pale cream colour is present [11].

Test for Terpenoids:

Salkowski's Test: 5ml of ethanol was added to 2ml of plant sample. Add 2 cc of mildly warmed and then cooled chloroform to this. Next, pour 1 cc of concentrated H_2SO_4 along the test tubessides. The formation of a grey-colored solution is indicative of terpenoids being present [12].

Test for Glycosides:
Legal Test: Add 1ml of the 0.3% sodium nitroprusside reagent and 2 drops of 10% sodium hydroxide to 5ml of the extract. Glycosides are indicated by a pink to red colour [13].

Test for Coumarins:
NaoH Test: Add a few drops of chloroform and 1ml of 10% NoaH to 1ml of sample. The presence of coumarins is indicated by the colour yellow [14].

Test for Anthocyanins:
HCl Test: Add 2ml of 2N HCl and 2ml of ammonia to 2ml of sample extract. Anthocyanins are indicated by a pinkish-red colour that turns bluish violet when ammonia is added [15].

***In-vitro* Anti-inflammatory Assays:**
Preparation of RBC Suspension for Membrane Stabilisation Assay: 4 ml of healthy human blood was drawn into heparinized centrifuge tubes from individuals who had not been administered non-steroidal anti-inflammatory medicines in around two weeks [16]. Then it is centrifuged for about 10minutes at 3000rpm. The supernatant containing serum is eliminated, and the RBC content is then gathered. Three equal washes of normal saline solution are performed on the RBC. Blood volume was calculated. The supernatant is preserved. Currently, 10% v/v suspension of normal saline is used to reconstitute RBC. For 30 minutes, the resulting suspension was incubated at 56°C. Centrifugation is then carried out at 2500 rpm for 5 minutes. Spectrophotometry at 560 nms is used to measure the absorbance of the supernatant after it has been collected. Saline is utilised as a good control. Calculated percentage inhibition is represented on a graph [17].

% inhibition = 100× (Absorbance of sample) / (Absorbance of control) × 100

Heat Induced Haemolysis: There was evidence of heat-induced haemolysis in these plant extracts. 1ml of a test sample made up of various plant extracts and 1ml of a 10% RBC solution make up the reaction mixture. The control test tubes received 1 ml of normal saline addition [18]. Phosphate buffer is added, and the pH is set to 7.4. In a shaking water bath, incubate test tubes for 20 minutes at 54°C. Centrifuge the test tubes for three minutes at 2500 rpm after cooling them. It is mentioned that an ultraviolet spectrophotometer reads an absorbance at 540nms [18]. Calculated is the percentage of haemolysis inhibition. Samples are shown on the x-axis, while percentage inhibition measurements are plotted on the y-axis [19].

% inhibition = (Absorbance of control – Absorbance of sample) /(Absorbance of control) × 100

Albumin Denaturation: Utilizing denaturation of albumin, all of the obtained plant extracts anti-inflammatory properties were researched.5ml reaction mixture contains 1ml of plant extracts, 1ml of 1% bovine albumin, and 3ml of pH-adjusted phosphate buffer solution. Boiling water bath at 37 °C for 20minutes of incubation later heated in a water bath for 10 minutes to 70°C. The proteins were denaturized by placing the mixture in a water bath for 10minutes at 70 °C [20]. After cooling, a U.V spectrophotometer was used to measure the turbidity at 660 nms. As a positive control, phosphate buffer solution is employed. Measured was percentage inhibition [21].

% inhibition = (Absorbance of control – Absorbance of sample) / (Absorbance of control) × 100

Proteinase Inhibitory Activity: The selected plant samples' protein inhibitory activity was examined. Trypsin and Tris HCL buffer were included in the reaction mixture that was added to these plant extracts. For 5minutes, at 37°C the reaction mixture was incubated. The reaction mixture was then given 1ml of 0.8% Casein, and it was allowed to sit at 37°C for 20minutes. To terminate the process, add 2ml of 70% perchloric acid [22]. Centrifuge for three minutes at 2500 rpm. The absorbance of the supernatant was measured at 210 nm in comparison to a buffer containing a blank. It was estimated how much of the proteinase inhibitory activity was inhibited [23].

% inhibition = (Absorbance of control – Absorbance of sample) / (Absorbance of control) × 100

Hypotoxicity Induced Haemolysis: These twelve particular plant extracts caused hemolysis when exposed to hypotoxicity. In test tubes, up to 1ml of plant extract is used. Add 1ml of the phosphate buffer solution to this. 2 ml of hyposaline solution should be added. 0.5ml of HRBC suspension was then added to this. As a normal medication, 1000 mg of diclofenac sodium was added. 30 minutes at

37°C of incubation. Centrifuged for five minutes at 3000rpm [24]. Supernatant with haemoglobin content was tested against a blank sample using a UV spectrophotometer at 540nms. Estimated proportion of hemolysis [25].

% inhibition = 100 × (Absorbance of sample) × 100

RESULTS:

Phytochemical Analysis:

TABLE 1: PHYTOCHEMICAL TEST ON TWELVE PLANT LEAF EXTRACTS

Test	Gloriosa Superba	Syzygium cumini	Canthium parviflorum	Holarrhena Antidysenterica	Costus	Polyalthia longifolia	Tabernae montana divaricata	Ficus	Tecoma stans	Tagetus erecta	Solanum melongena	Solanum lycopersicum
Phenol	+	+	+	+	+	--	+	--	+	+	--	+
Saponins	+	+	+	--	+	+	+	--	+	+	+	+
Tannins	--	+	--	+	--	+	--	+	--	+	+	+
Flavonoids	--	+	--	--	--	+	+	+	+	+	--	+
Quinones	--	+	--	--	+	+	--	--	--	+	+	+
Alkaloids	--	+	--	--	--	--	+	+	+	+	+	--
Terpenoids	+	+	+	+	--	+	+	--	+	--	+	--
Glycosides	--	+	--	--	--	+	--	--	+	+	+	--
Coumarins	+	+	+	+	+	--	+	+	+	+	+	--
Anthocyanins	--	+	--	--	--	--	--	--	--	--	+	+

Fig. 1: here + indicates positive response for that particular activity. Indicates negative response for that particular activity.

In-vitro Anti-inflammatory Assays:

Membrane Stabilization Assay:

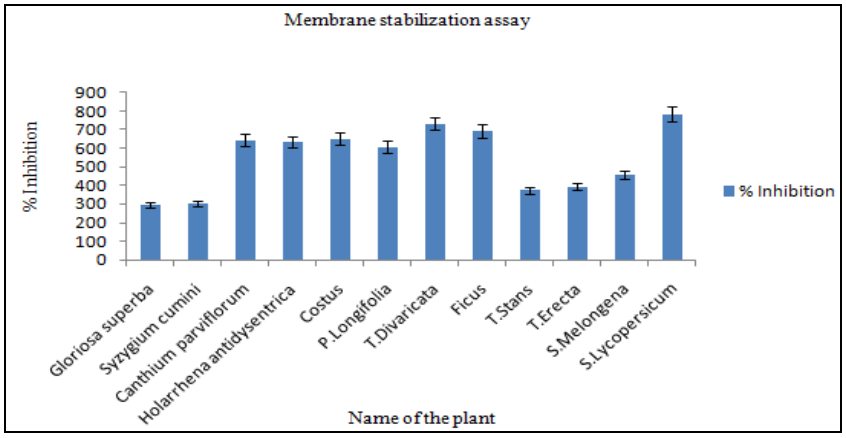

FIG. 1: MEMBRANE STABILIZATION ASSAY WAS PERFORMED, TAKING THE NAME OF THE PLANT ON THE X-AXIS AND PERCENTAGE INHIBITION ON Y-AXIS

Heat-Induced Haemolysis:

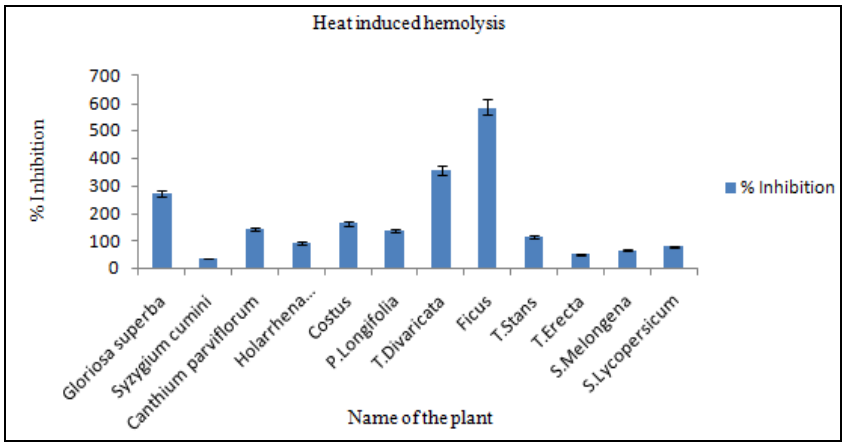

FIG. 2: HEAT-INDUCED HEAMOLYSIS IS ESTIMATED. ON X-AXIS THE NAME OF THE PLANT IS TAKEN, AND ON Y-AXIS PERCENTAGE INHIBITION IS TAKEN

Albumin Denaturation:

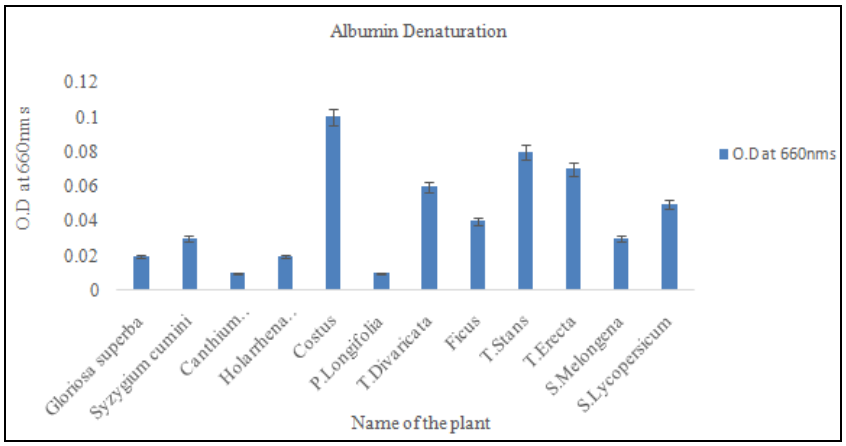

FIG. 3: DENATURATION OF PROTEIN IS DONE TAKING THE NAME OF THE PLANT ON THE X-AXIS AND OPTICAL DENSITY READINGS AT 660NMS ON THE Y-AXIS

Proteinase Inhibitory Activity:

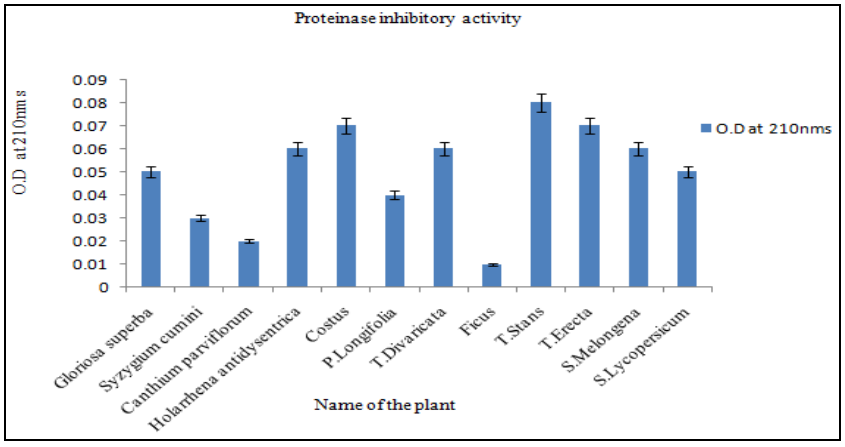

FIG. 5: PROTEINASE INHIBITORY ACTIVITY IS PERFORMED. ON X-AXIS THE NAME OF THE PLANT IS TAKEN, AND ON Y-AXIS, OPTICAL DENSITY READINGS AT 210NMS IS TAKEN

Hypotoxicity Induced Haemolysis:

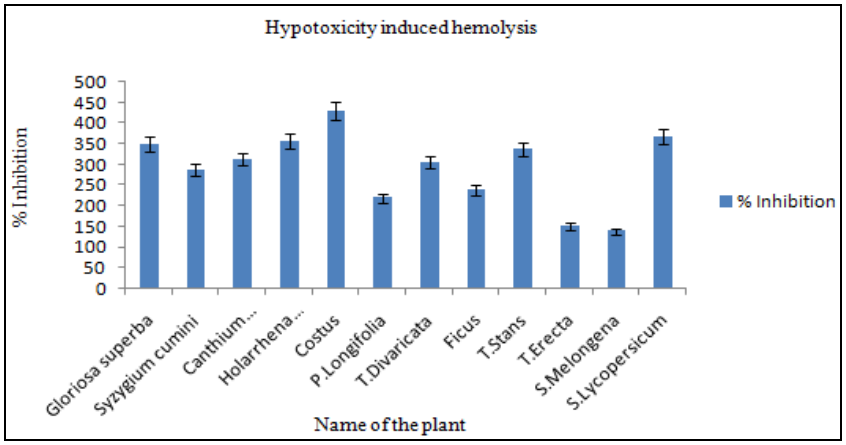

FIG. 6: HYPOTOXICITY-INDUCED HAEMOLYSIS IS ESTIMATED TAKING NAME OF THE PLANT ON THE X-AXIS AND PERCENTAGE INHIBITION ON Y-AXIS

DISCUSSION: Since from many years plants and its products are used to treat many life harming diseases. But even today there are some unexplored compounds from plants that can treat human

diseases. Here all about twelve plants were identified and conducted phytochemical analysis and anti-inflammatory assays on *Gloriosa superba, Syzyniumcumini, Canthium parviflorum, Holarrhena antidysentria, Costus, Polyalthia longifolia, Tabernaemontana divaricata, Ficus, Tecoma stans, Tagetuserecta, Solanum melongena, Solanum Lycopersicum*. At first phytochemical screening is done, out of which *Sygyzium cumini, Gloriosa superba, Canthium parviflorum, Solanum melongena, Tagetuserecta, Tabernaemontana divaricate* have shown activity for maximum phytochemicals. Five *in-vitro* anti-inflammatory assays were conducted. Twelve different plant extracts were tested in a membrane stabilisation assay for their ability to reduce inflammation; out of these, *Canthium parviflorum*, *Holarrhena antidysentria*, Costus, *Polyalthia longifolia*, Tabernaemontana Divaricata, Ficus, and *Solanum lycopersicum* demonstrated the highest levels of activity. These findings support membrane stability as a second mechanism underlying their anti-inflammatory activity. This impact might prevent neutrophil lysosomal content from being released at the site of inflammation. *Gloriosa superba*, *Tabernaemontana divaricata*, and Ficus extracts were successful in preventing heat-induced hemolysis.

One frequently stated reason for inflammation is protein denaturation. The ability to extract protein denaturation was investigated as a component of evaluation of the mechanism of the anti-inflammation effect. It was successful in preventing albumin denaturation brought on by heat. Fresh leaf extract from *Syzynium cumini*, Costus, *Tabernaemontana divaricata*, Ficus, Tecoma stans, Tagetuserecta, and *Solanum melongena* showed the greatest level of inhibition.

Significant anti-proteinase activity was discovered in the fresh plant extracts' leaves. Fresh leaf pbs extracts from *Tecoma stans*, Costus, Tagetuserecta, *Holarrhena antidysentria*, *Solanum melongena*, *Gloriosa superba*, *Tabernaemontana divaricate*, *Solanum lycopersicum*, and *Polyalthia longifolia* were shown to have the highest levels of inhibition. The erythrocyte membrane is significantly protected from lysis brought on by a hypotonic solution, according to research on the plant extracts of *Gloriosa superba, Syzyniumcumini, Canthium parviflorum, Holarrhena antidysentria*, *Costus*, *Tabernaemontana divaricate*, *Tecoma stans,* and *Solanum lycopersicum*. Diclofenac sodium provided a lot of protection against the harmful effects of hypotonic solution.

CONCLUSION: Among these twelve plants studied in this research investigation, almost all the plants have shown positive results for phytochemical analysis. *Gloriosa superba* has shown the highest activity against Heat-induced haemolysis, Proteinase inhibitory activity and Hypotoxicity induced haemolysis. *Syzynium cumini*has shown high activity against Albumin denaturation, Proteinase inhibitory activity and Hypotoxicity induced haemolysis. *Canthium parviflorum* has exhibited highest activity against Membrane stabilization assay and Hypotoxicity induced haemolysis. *Holarrhena antidysentria* has shown highest activity for Membrane stabilization assay, Proteinase inhibitory activity and Hypotoxicity induced haemolysis. *Costus* has shown high activity against Membrane stabilization assay, Albumin denaturation, Proteinase inhibitory activity and Hypotoxicity induced haemolysis. *Polyalthia longifolia* has shown high activity for Membrane stabilization assay and Proteinase inhibitory activity.

Tabernaemontana divaricate has exhibited high activity for Membrane stabilization assay, Albumin denaturation, Proteinase inhibitory activity, Heat induced haemolysis and Hypotoxicity induced haemolysis. *Ficus* exhibited high activity for Membrane stabilization assay, Heat induced haemolysis and -Hypotoxicity induced haemolysis. *Tecoma stans* has shown highest activity against Albumin denaturation, Proteinase inhibitory activity and Hypotoxicity induced haemolysis. *Tagetuserecta* has shown high activity for Albumin denaturation and Proteinase inhibitory activity. *Solanum melongena* has shown highest activity against Proteinase inhibitory activity. *Solanum Lycopersicum* has shown high activity for Membrane stabilization assay, Proteinase inhibitory activity and Hypotoxicity induced haemolysis. It is concluded that *Gloriosa superba, Syzynium cumini, Canthium parviflorum, Costus, Tabernaemontana divaricata, Ficus, Tecoma stans* and *Solanum Melongena* rich in phytochemical content and anti-inflammatory properties indicating its high

medicinal values. Hence, we have decided to conduct further studies on the characterization of bioactive compounds with these plants as they show potential activities among all the other sources tested.

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CONFLICTS OF INTEREST: Nil

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