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Anti-Inflammatory Activities of Some Medicinal Plants

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Abstract- Inflammation is classically regarded as a feature of innate immunity. It is characterized by redness, swelling at the site of injury and pain due to the influx of neutrophils, leukocytes and quickly followed by the influx of monocytes. Monocytes mature to inflammatory macrophages, proliferate and eventually alter the cellular function. The initiation and rapid onset of inflammation is mediated through receptors, unlike in adaptive immunity and involves 4 stages- a trigger by the stimulant (sterile/ infection), a radar that recognises the danger (receptors), signal transduction to the nucleus to initiate production of various mediators for combating the damage and clearance of the stimuli. The inflammatory response is the coordinate activation of signalling pathways that regulate inflammatory mediator levels in resident tissue cells and inflammatory cells recruited from the blood. Inflammation is a common pathogenesis of many chronic diseases, including cardiovascular and bowel diseases, diabetes, arthritis, and cancer (Lugrin et al., 2014).

Keywords- Pharmacology activities, Phytochemical, Folklore plants, Medicinal values in vitro as well as in vivo studies. Traditional Chinese Medicine

I. INTRODUCTION

Plant-derived phytochemicals have emerged as novel anti-inflammatory agents. Due to the diversity of chemical phytochemicals, they include many therapeutic indicators for cancer, inflammation, and non-neurodegenerative diseases, and have been a productive source of lead-based compounds for novels. In fact, most active drugs work with more flexibility than just one protein. In recent years, the pharmaceutical industry has faced challenges such as rising drug development costs, high failure rates, and increasing competition for guaranteed targets, as well as the search for new intentions and drug-based products (Kang, 2021).

bioactives as well as a possibility for combinatorial chemistry on the novel pharmacophores (Havsteen, 2002).

The present investigation was designed to evaluate the phytochemical components of Euphorbia tithymaloids, Luffa cylindrica, and Coccinia grandies and its anti –Inflammatory Activity

II. MATERIALS AND METHODS

1. Study of Selected Plants

Collection and Authentication of plant material
The herbarium of Artocarpus heterophyllus have been submitted to Unique College, Bhopal for its authentication. It has been identified and authenticated by Prof. Tenguriya sir head, Department of --botany College, Bhopal with Voucher Reference No.01.

2. Processing of Plant

Plant parts used for study was collected and washed under running tap water. All plant material kept in shade and allows it to dry. Powder of dry

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plant material was prepared by using a blender. Powder form plant material was stored in storage place with strong air for further use.

S. No	Botanical name	Part used
1.	Euphorbia tithymaloidus	Leaves
2	Coccinia grandies	Leaves
3	Luffa cylindrical	Leaves

Physicochemical Studies

The physicochemical parameters such as Total ash, Water Soluble Ash, Acid insoluble Ash, Solvent extractive value, Loss on drying of plant Euphorbia tithymaloidus, Coccinia grandies and Luffa cylindrica was carried out(Khandelwal K.R. 2008).

Total Ash

2g of air-dried powdered part of selected plant was accurately weighed and put into the tared silica crucible. The crucible was supported on a pipe-clay triangle placed on a ring of retort stand. Then, it was heated with a burner, using a flame about 2cm high and supporting the crucible about 7 cm above the flame heat till vapours almost ceased to be evolved; then it was lowered and heated more strongly until all carbon was burnt off. It was then cooled in a desiccator. The ash thus produced was weighed and the percentage of total ash with reference to the air-dried sample of the crude drug was calculated.

Total ash value (%) = (wt. of total ash/wt. of total crude material) *100

Water Soluble Ash

The ash obtained while calculating total ash value was washed using 25 ml of distilled water into 100ml beaker. It was boiled for 5 minutes and then filtered through an ashless filter paper. The residue thus obtained was washed twice with hot water. The crucible was ignited, cooled and weighed. The filter paper containing the residue was put into the crucible which was then heated gently until vapours ceased to be evolved and then more strongly until all carbons has been removed. After the ignition, it was cooled in a desiccator. The residue was weighed and water-soluble ash was calculated by subtracting the weight of residue from weight of total ash. The % of water-soluble ash was then calculated with reference to air-dried powdered drug.

$$\% \text{ Water soluble ash value} = \frac{\text{Wt. of total ash - Wt. of water insoluble ash}}{\text{Wt. of crude drug taken}} \text{ X } 100$$

Acid Insoluble Ash

First of all, the total ash was washed using 25ml of 2N HCl into 100ml beaker. It was then boiled for 5 minutes and then filtered through an ashless filter paper. The residue thus obtained was washed twice with hot water. The crucible was ignited, cooled and weighed. The filter paper containing the residue was put into the crucible which was then heated gently until vapours ceased to be evolved and then more strongly until all carbons have been removed. After the ignition, it was cooled in a desiccator. The residue was weighed and acid- insoluble ash was calculated by subtracting the weight of residue from weight of total ash. The % of acid insoluble ash was then calculated with reference to air-dried powdered drug

$$\% \text{ Acid insoluble ash value} = \frac{\text{Wt. of acid insoluble ash}}{\text{Wt. of crude drug taken}} \text{ X } 100$$

Loss on Drying

Firstly 3.0 gm of powdered part of selected plant was weighed accurately and put in a tarred porcelain dish which was earlier dried at 105˚C using hot air ovens at constant weight. Using the difference in weight, the percentage loss of drying with reference to the air-dried substance was calculated.

Extraction of Selected Plants Using Different Solvent

The collected plant material was extracted in Soxlet apparatus by using continuous hot percolation method. Powdered material of Euphorbia tithymaloidus, Coccinia grandies andLuffa cylindrica was placed in thimble of soxhlet apparatus. The process of extraction was performed at 60°C using petroleum ether as non-polar solvent. The re-extraction of dried exhausted plant material was carried out with pet ether, ethyl acetate, and 70% methanolsolvent. The process of soxhlation was continued till no visual colour change was observed

in siphon tube for each solvent. The process of extraction was completed when there was no residual solvent, when evaporated. The plant extract obtained was evaporated using rotary vaccum evaporator (Buchitype) at 40 C. The physical characteristics (colour and odour) of prepared extract were observed and packed in air tight container and labeled till further use (Kokate., 1994, Mukherjee et al., 2002). Dried extract was weighed and percentage yield for each extract was determined using formula:

Qualitative Phytochemical Testing of Plant Extract

Phytochemical screening was performed using standard procedures to identify the presence and absence of different phytochemical constituents in Euphorbia tithymaloidus, Coccinia grandies andLuffa cylindrica was carried out extracts by using standard procedures (Kokate et al., 2006, Kokateet al., 2004). The extracts prepared in Petroleum ether, Methanol and Ethyl acetate were subjected to following tests:

Tests for Carbohydrates

Molisch Test

To 1ml of extract, 2-3 drops of alcoholic α-naphthol solution was added. Sulphuric acid was added along the side of the test tube till the appearance of purple ring at the junction of two liquids. It confirms the presence of carbohydrates in the test sample.

Fehling Test

The Fehling solution A and B was added to 1ml of extract and heated on a water bath for few minutes. The development of brick red precipitate was observed.

Benedict's Test

The mixture of equal volume of Benedicts reagent and extract were mixed in a test tube and heated in water bath for 5-10 minutes. Depending on the presence of amount of reducing sugar present in the test solution the colour of solution changes to green, yellow or red. This change in the colour of solution indicate the presence of reducing sugar.

Barfoed's Test

To 1ml of plant extract, few drops of Barfoed's reagent was mixed in a test tube and heated on water bath for 2minutes. The red colour was appeared due to the formation of cupric oxide indicates the presence of monosaccharide.

Test for Alkaloids

All the test extracts were first treated with dil. hydrochloric acid separately and filtered. The filterate of all the test extracts were exposed to following tests:

Mayer's Test

To 1-2ml of extract filtrate, equal amount of Mayer's reagent was added alongside of tube. The presence of alkaloids was confirmed after formation of white or creamy precipitate.

Hager's Test

To 1-2ml of extract filtrate, equal amount of Hager's reagent was added in a test tube. The presence of alkaloids was confirmed after formation of yellow coloured precipitate.

Wagner's Test

To 1-2ml of extract filtrate, equal amount of Wagner's reagent was added in a test tube. The presence of alkaloids was confirmed after formation of reddish-brown coloured precipitate.

Test for Flavonoids

Lead Acetate Test

Few drops of lead acetate solution were added to the plant exreact. Formation of yellow precipitate may indicate the presence of flavonoids.

Alkaline Reagent Test

Few drops of sodium hydroxide added to the plant extract in a test tube. Formation of intense yellow colour, which becomes colour less on addition of few drops of dilute acid which indicates the presence of flavonoids.

Shinoda Test

To the plant extract, 5ml ethanol (95%) was added. To this mixture few fragments of magnesium turning was added followed by drop wise addition

of concentrated hydrochloric acid. Formation of pink colour indicates the presence of flavonoids.

Test for Glycoside

Borntrager's Test

In 3 ml of extract, sulfuric acid was added, boiled for 5 minutes and filtered. In cold filtrate, an equal amount of benzene or chloroform was added and stirred considerably. The organic solvent layer was separated and ammonia was added to it. The formation of a reddish-brown color in the ammonical layer indicates the presence of anthraquinone glycosides

$$\%\ \text{Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

Legal's Test

1 ml of extract dissolved in pyridine. 1 ml of sodium nitropruside solution was added alkaline using a 10% sodium hydroxide solution. The formation of pink in the blood-red color indicates the presence of cardiac glycosides.

Keller-Killianitest

In 2 ml of extraction, 3 ml of glacial acetic acid and one drop of 5% ferric chloride were added to the test tube. Carefully add 0.5 ml of concentrated sulfuric acid to the side of the test tube. The formation of blue color in the acetic acid layer indicates the presence of cardiac glycosides.

Biuret Test

The extract was treated with 1 ml of sodium hydroxide solution in a test container and burned. Add a drop of 0.7% copper sulfate solution to the above mixture. The formation of violet or pink indicates the presence of protein.

Ninhydrin Test

3 ml of ad was warmed with 3 drops of Ninhydrin solution in 5 bath water for 10 minutes. The formation of a blue color indicates the presence of amino acids.

Tests for Saponins

Foam Test

1ml of plant extract was dissolved in 20 ml of distilled water and allows shaking for 15 min in graduated cylinder. Formation of persistent foam around 1cm layer indicates the presence of saponons.

Tests for Terpenoids and Steroids

Salkowski's Test

The test sample was treated equal amount of chloroform and filterd. Few drops of concentrated sulphuric acid was added to the collected filtrate, shaken and allow to stand. The fprmation of the golden yellow layer at bottom indicates the presence of triterpenes and if the lower layer turns red, it shows the presence of sterol.

Libermann-Burchard's Test

The plant extract was treated with chloroform and few drops of acetic anhydride was added, boiled and cooled. The solution was taken in the test tube and concentrated sulphuric acid was added through the sides of the test tube. The formation of brown ring at the junction of two layers was observed. The change in the colour of upper layer changed to green, it indicates the presence of steroids and if colour changes to deep red colour indicates the presence of triterpenoids.

Tests for Tannins and Phenolic Compounds

Ferric Chloride Test

Little amount of plant extract was dissolved in distilled water and 2ml of 5 % ferric chloride solution was added. Formation of blue, green or violet colour showed the presence of phenolic compounds.

Lead Acetate Test

Little amount of plant extract was dissolved in distilled water and few drops of lead acetate solution were added. Formation of white precipitate confirms the presence of phenolic compounds.

Isolation of Bioactive Compounds of Selected Plants by TLC and Column Chromatography

In order to identify and separate the components of the extract of Euphorbia tithymaloidus and Cocciniagrandies two Chromatographic methods were used. Methods involved are Thin-Layer-Chromatography (TLC) and Column Chromatography (CC).

Thin Layer Chromatography (TLC)

The pet. ether, ethylacetate and methanol extract obtained from the Euphorbia tithymaloidus and Coccinia grandies were subjected to purification process by chromatographic techniques. TLC was produced with the aim of identifying the individual substances in a mixture and also testing for purity or for separation of mixtures (Trease and Evans, 1989; Kokateet al., 2006).

Thin layer chromatography (TLC) is a simple and inexpensive analytical technique that can quickly and efficiently separate quantities of less than ten micrograms of material. TLC has many applications in the organic laboratory. TLC is used for the rapid analysis of reagent and product purity, or to quickly determine the number of compounds in a mixture. Also, by comparing an unknown compound's behaviour to the behaviours of known standard compounds, mixture compounds can be tentatively identified. TLC is performed on a sheath of glass, plastic or aluminum foil which is coated with a thin layer of adsorbent material, usually silica gel or cellulose. This layer of adsorbent is known as stationary phase. The plate or sheath is placed in a chamber containing a small amount of solvent which acts as mobile phase. The height of the solvent front and center of spots were measured in the form of Rf value. The Rf value indicates the position at which a substance was located in the chromatogram. A TLC experiment has three general stages: spotting, developing, and visualizing. In the present study TLC was done for crude extract, pet.ether, ethylacetate and methanol fractions of Euphorbia tithymaloidus and Coccinia grandies to find out the probable number of compounds present in them.

Spotting a Plate

The origin is marked, usually by drawing a thin line across the bottom of the plate with a pencil. The methanol extract was dissolved in a volatile solvent. A glass capillary tube was used to apply a small amount of sample solution into the plate, keeping the sample in as small an area as possible. With practice, spots with diameters of 1-2 mm were produced.

Developing a Plate

To develop the chromatogram, a piece of filter paper was placed along the walls of the developing chamber which contains a shallow layer of the appropriate eluent. The paper acts as a wick that adsorbs the eluent and ensures that, when the chamber was closed, its atmosphere was saturated with eluent vapour, minimizing evaporation from the plate. The spotted plates were placed into the chamber; the origin marked on the plate must be higher than the level of the eluent, to prevent the sample from dissolving from the plate into the eluent layer. When the eluent reached a point approximately 5 mm from top of the plate, the plates were removed from the chamber. The point that the eluent has reached is called the eluent front (or solvent front) and was immediately marked with a pencil.

Visualizing the Compound

Upon development, a successful separation of coloured compounds will reveal distinct spots, indicating that the mixture compounds have separated. To make separated colorless compounds observable to the eye, the spots are treated in some way to make them visible. The process is called visualization. In the present study a number of developing solvent systems were tried during the study. The most informative and satisfactory resolution was taken as final solvent system. These solvent system were:Ethyl acetate: Formic acid: water (6: 6: 1) and Methanol: water (9:1). TLC plates were observed under visible light, short wave UV light and long wave UV light. After development of plates, they were air-dried and number of spots, colour and Rf values were recorded.

Spot Distance from Origin

Column Chromatography

Column chromatography is one of the most regularly used techniques in the isolation of natural plant constituents. In principle, plant constituents are distributed between the solid phase (for example silica gel or Sephadex) and the mobile phase, which comprises an eluting solvent.

In silica gels the separation of compounds from each other in an extract is based on a number of

factors including the polarity of compounds, hence compounds are eluted from the column with solvent systems of differing polarity. Silica gels constitute polar ends which interact strongly with polar compounds and they are eluted later from the column (Iscan et al., 2002). In Sephadex gel filtration the separation of constituents in an extract depends on the size of the molecules. Constituents with a small molecular weight interact strongly with the matrix of the gel and tend to move slowly through the gel and they are eluted later while the large molecular weight constituents are eluted early because they move fast through the column.

Protocol for Column Chromatography

The methanol extract was concentrated by distilling off the solvent and evaporated to dryness. The methanol extract (5 gm) was suspended in Petroleum ether and methanol and then resulting solutions were concentrated and were eluted with the solvent of increased polarity i.e. Non-polar-polar – highly polar.

Preparation of Column for isolation of phytoconstituents adsorbent silica gel (60-120 mesh)

Activation	110 °C for 1 hour
Length of column	43 cm
Diameter	3.5 cm
Length of adsorbent	25 cm.
Rate of elution	5-10 drops/min.

Glass column was packed by wet filling. The slurry of adsorbent (silica gel; 230-400 mesh) was prepared by mixing the adsorbent in the petroleum ether and used as stationary phase. It was then poured into glass column (43cm x 3.5 cm) (having sintered glass disc at its bottom) and allowed to settle. The air entrapped was removed by stirring with glass rod. A small amount of sand was kept at top the column to provide the latter a flat base. Excess of solvent was run off until the level of mobile phase fell to one cm just above the top of the sand layer.

Preparation of Sample and Loading

The methanol extract of Euphorbia tithymaloidus and Coccinia grandies, were subjected to silica gel (230-400 mesh) column (43cm x 3.5 cm) chromatography for the isolation of phytoconstituents and elution was carried out in selected solvent system as mention in result section.

Figure 1: Column Chromatography showing Separation of bioactive constituents

Separation of bioactive constituents from methanol extracts dissolved in a minimum volume of pet. ether, adsorbed on silica gel (230-400 mesh), dried and applied on the column to separate possible phytoconstituets. Petroleum ether insoluble part was eluted gradiently with ethyl acetate and methanol mixtures. At uniform interval, the eluents (each of five ml) were collected and the progress of separation was monitored by thin layer chromatography (TLC) (silica gel G 60 F254 TLC plates of E. Merck, layer thickness 0.2mm) using solvent system, Methnaol: water (9:1) for methanolic extract. Presence of no. of spots was considered as criteria for selection of fraction for isolation of pure compound.

Spectral Analysis of Isolated Component Using UV-Vis Spectroscopy

UV-Vis spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. This property is influenced by the sample composition, potentially providing information on what is in the sample and at what concentration.

To identify the UV-Vis spectrum profile, the methanolic extract of Euphorbia tithymaloidus and Coccinia grandies, were scanned in the wavelength ranging from 210–800 nm on Systronic UV-Vis spectrophotometer model. Sample was extracted 3 times with 50% aqueous methanol, after optimization of extraction conditions. The characteristic peaks were identified to confirm the different bioactive copmonents present in the sample extracts. The peak values of the UV-Vis were documented (Skoog et al., 2007; Pavia 2011).

NMR Spectroscopy

NMR spectroscopy is a research technique that utilizes the magnetic properties of some atomic nuclei. It evaluates the physical and chemical properties of atoms or the molecules in which they are contained. NMR of isolated component of plant Euphorbia tithymaloidus and Coccinia grandies was carried out by using a Bruker 600 MHz instrument equipped with a cryogenic probe. Sample temperatures were stabilized at 298 K. The deuteriome-thyl13C signal and the residual 1H signal of the solvent (d6-DMSO) were used as secondary references (δ 39.5 and 2.5 from TMS, respectively) (Skoog et al., 2007; Pavia 2011).

In Vitro Antioxidant Activity of Plant Extract

DPPH Radical Scavenging Assay

The molecule of 1,1-diphenyl-2-picrylhydrazyl(α,α-diphenyl-β-picrylhydrazyl; DPPH:1) is characterised as a stable free radical by virtueof the delocalisation of the spare electron over themolecule as a whole, so that the molecules do notdimerise, as would be the case with most otherfree radicals. The delocalisation also gives rise tothe deep violet colour, characterised by an absorptionband in ethanol solution centred at about 517nm.

0.1mM solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol was prepared. To assess the scavenging ability on DPPH (0.1mM), solution of selected plant extract was prepared in methanol. Different concentration of solutions were prepared in the test tube from 20-100µg/ml. 1ml of prepared plant extract solution was mixed with 2 ml DPPH. The mixture was shaken vigorously and left to stand for 30 mins in the dark before measuring the absorbance at 517nm against a blank (Liyana-PathiranamandShahidi 2005).

Then the scavenging ability was calculated using the following equation:

$$I\% = 100\times (A\ blank - A\ sample/\ A\ blank)$$

Where, I (%) is the inhibition percent, a blank is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound.

In Vivo Anti-Inflammatory Activity

Caragenan Induced Paw Edema

Carrageenan-induced rat paw edema model is a suitable test for evaluating anti-inflammatory drugs, which has frequently been used to assess the antiedematous effect of the drug. Carrageenan is a strong chemical use for the release of inflammatory and proinflammatory mediators (prostaglandins, leukotrienes, histamine, bradykinin, TNFα, etc.) (A. L. Wills, 1969; B. Bhukya, et al., 2009).

Animals were divided into 6 groups. group I received Carrageenan 0.1 ml of 2% (w/v) along with saline, group III and IV received Euphorbia tithymaloidus and Coccinia grandies extract (200 mg/kg) orally for 10 days, group IV received Isolated fraction (30 mg/kg bw) for 10 consecutive days (orally). Group II received standard drug indomethacin (10 mg/kg) administered intraperitoneally 1 h before carrageenan suspension administration. The last dose was administered 60 min before the induction of inflammation. Subsequently, all animals received a subcutaneous injection of 0.1 ml of 1% (w/v) carrageenan solution in the plantar region of the right hind paw to induce edema. The paw volume was measured initially and then at 30 min intervals for up to 5h after the injection, using a vernier caliper.

III. CONCLUSION

In the present investigation, extraction of plants Coccinia grandies, Euphorbia tithymaloidus and Luffa cylindrica was carried out using different solvents and extract was used for evaluation of the

different properties like phytochemical components which are highly responsible for the antioxidant as well as anti-inflammatory activity of the plant. According to the results of the present investigation it was found that different phytochemicals like phenols, flavonoids, alkaloids, terpenoids, saponins, tannins, protein and amino acids, glycosides and terpenoids are present in the selected plants. The presence of different phytochemical components is responsible for the anti-oxidant property of the plants.A broad range of roles of secondary plant metabolites has been identified for several inflammatory cells and for a large number of inflammatory mediators in important pathologies not previously known to be linked to inflammation, such as obesity, atherosclerosis, and cancer. According to the results Coccinia grandies and Euphorbia tithymaloidus showed most important phytochemical components which are the proceed for the chromatography to find out the specific elute and then proceed for the different spectrophotometric like UV- VIS, and NMR spectroscopy. NMR spectroscopyis the preeminent technique for determining the structure of organic compoundsand is the only one for which a complete analysis and interpretation of the entire spectrum is normally expected. It identifies the carbon-hydrogen framework of an organic compound. The active components from Coccinia grandies and Euphorbia tithymaloidus were characterized using several spectral analys is like UV- VIS, and NMR spectroscopy. The results of the respective study showed the presence of the Lupeol and Luteolin in Coccinia grandies and Euphorbia tithymaloidus respectively. An anti-oxidant potential of the selected plats was determined by using DPPH radical scavenging activity and it was found that all plats showed significant anti-oxidant activity.

In vivo studies was carried out to evaluate the anti-inflammatory activity of sected plants by using Carrageenan-induced paw oedema model in experimental animals. Carrageenan-induced paw oedema is a well-established in vivo model of inflammation and has been commonly used to evaluate the anti-oedematous effect of natural products. According to the given results it was observed that isolates were more effectiveas compared to the plant extract.Isolate from Euphorbia tithymaloidus was found to be more effective as compared to the isolate of after 5 hr of time interval.Some studies have indicated that inflammatory effect induced by carrageenan is correlated with free radicals (Dawson J et al., 1991). Carrageenan-induced inflammatory response has been associated with neutrophil infiltration and with the production of neutrophil-derived free radicals, for instance superoxide, hydroxyl radicals and hydrogen peroxide, as well as due to the release of other neutrophils-derived mediators (Lu TC et al., 2011).

Thus, after a study of the investigation about phytochemical study, anti-oxidant study and in vivo anti-inflammatory study, we are able to determine the qualitative information which helps to identify and determine the isolate from the selected plants as well as the effect of isolated component in in vivo studies. Hence it can be concluded that plant Coccinia grandies and Euphorbia tithymaloidus may use as an anti-inflammatory agent in different therapeutic treatment.

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