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ANTI-INFLAMMATORY AND ANTIHYPERCHOLESTEROLEMIC ACTIVITY OF ROSA CENTIFOLIA, CORIANDRUM SATIVUM AND CYNODON DACTYLON

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ABSTRACT: Hypercholesterolemia is one of the major conditions that are prevalent in today's world, the significant causes being unhealthy food habits and lifestyle or abnormal genetic conditions. HMG CoA reductase is an important rate-limiting enzyme in the mevalonate pathway that synthesizes cholesterol. Biosynthesis of cholesterol in the liver can be reduced significantly by inhibiting HMG CoA reductase. Statins are the drugs used for the treatment of hypercholesterolemia. Due to the undesirable effects caused by synthetic statins, there is a need for the development of natural HMG CoA reductase inhibitors that are of plant origin. This study aimed to investigate the antiinflammatory and HMG CoA reductase inhibitory effects of different hydro alcoholic plant extracts on the enzyme HMG CoA reductase activity obtained from Gallus gallus domesticus liver. Our study showed that the hydroalcoholic extract of Rosa centifolia exhibited the maximum inhibition of the enzyme under analysis with a ratio of 1.4117 and hence was subjected to solvent-solvent partition for the partial isolation of the inhibitory compounds. The result of this study suggests that *Rosa centifolia* extract has potential antihypercholesterolemic activity.

INTRODUCTION: Hypercholesterolemia is a condition characterized by elevated cholesterol levels in the blood of animals caused by various factors that are environmental or genetic ¹. The optimum concentration of serum cholesterol is less than 200 mg/dL in humans. Serum cholesterol levels greater than the optimum leads to hypercholesterolemia and in turn causes heart associated diseases ². Cholesterol is insoluble in water is transported within small protein particles as lipoproteins such as low density and high-density lipoproteins. Elevated levels of low-density lipoprotein cause hypercholesterolemia, as the main component of LDL is cholesterol.



The risk of stroke due to hypercholesterolemia can be reduced by about 29% by decreasing the activity of HMGCR which in turn reduces the cholesterol levels in the blood ^{3, 4}. HMG CoA reductase is a rate-limiting enzyme in the mevalonate pathway of cholesterol biosynthesis in humans catalyzing the conversion of 3-hydroxy-3-methylglutaryl CoA (HMG CoA) to mevalonate. HMG CoA reductase can be categorized into two classes: Class 1, found in eukaryotes and Class 2, found in prokaryotes ⁵.

Transcription and translation of the enzyme are said to increase when the levels of the mevalonate pathway products are quite low in the blood ⁶. The enzyme can also be regulated by phosphorylation that is achieved by protein kinases that are AMP-activated, thereby reducing the HMG CoA reductase activity ^{7, 8}. Statins are drugs that can reduce the lipid levels in the blood ⁹. They inhibit HMG CoA reductase activity thereby preventing the formation of mevalonate from HMG CoA by acting as competitive inhibitors ^{10, 11}.

Statins have an HMG like a moiety due to which they bind competitively to HMG CoA reductase at the active site, inhibiting it ¹². In considerable cancer cell types, the inhibition of HMG CoA reductase leads to the arrest of cell growth and hence the death of the cell ^{13, 14}. Rosa species have been reportedly used in aromatherapy to reduce the post operative pain in children ¹⁵. The oil of Rosa centifolia has been reported to have antiseptic, aphrodisiac, astringent, anti-arthritic and anti-mutagenic properties ^{16, 17, 18}. A recent study has also highlighted the analgesic and anti-anxiety effects of rose oil in humans ¹⁹. Coriander is a common herb that is known for its aromatic seeds and foliage. It is native to Europe and the Mediterranean region. The sample has also been reported to possess antibacterial and antifungal properties against selected micro-organisms like Staphylococcus Bacillus subtilis, aureus. Salmonella typhi, and Candida species ²⁰. Recent studies have also demonstrated the anti granuloma property of the herb²¹.

Cynodon dactylon mostly known by common names Bermuda grass, Bahama grass, durva grass, *etc.*, is a grass native to the Middle East. The Bermuda grass is used for gastrointestinal disorders, kidney problems, and urinary infections and also as a blood purifier ²². The plant sample is also used for treating syphilis, piles, and dropsy and wound infections. It is used as an ethnomedicine to carbuncles, hypertension, and gout ^{23, 24}. From the ethnopharmacological point of view, the plant is used in the management of wounds and is used as hemostatic agent ²⁵.

According to a study by WHO, a significantly high percentage of the population in developing countries is dependent on traditional medicine as their primary health care ²⁶. *Rosa centifolia*, *Coriandrum sativum*, and *Cynodon dactylon* were chosen for this work owing to the increasing demand for herbal products. This study focuses on the anti-hypercholesterolemic activity of the plant samples and also compares their anti-inflammatory effects.

MATERIALS AND METHODS:

Preparation of Sample Extract: The plant samples *Rosa centifolia, Coriandrum sativum,* and *Cynodon dactylon* were procured from K.R. market in Bengaluru by random selection. The samples were washed clean with distilled water and air dried. The 5% raw extracts of the plant samples were prepared by homogenizing the petals of *Rosa centifolia*, stems of *Coriandrum sativum* and leaves of *Cynodon dactylon* with distilled water, 50% methyl alcohol and chloroform separately using mortar and pestle. The crude extracts were filtered and centrifuged at high speed for 10 min and stored at 4 °C in an airtight container for further use.

Chemicals: The chemicals used were arsenate, bovine serum albumin, ferric chloride reagent, hydroxylammonium chloride, perchloric acid, phosphate buffer saline, sodium citrate, and sodium dodecyl sulphate.

Equipment: The types of equipment used were UV-visible spectrophotometer, weighing balance, cooling centrifuge and UV transilluminator.

Liver Tissue Source: The chicken liver was obtained from Karnataka Chicken Centre in Vasanthnagar, Bangalore. The chicken liver was cut into pieces of required weight after washing it with 0.9% saline.

In-vitro Anti-inflammatory Assay: The test sample extracts were screened for their anti-inflammatory activity by protein denaturation assay with a few modifications studied according to the protocol by Muzushima and Kobayashi²⁷. Aspirin (1 mg/mL) was used as the standard drug and 2% bovine serum albumin was used as the control. Test samples with BSA were incubated at 51 °C for 20 min to induce denaturation. The turbidity was measured spectrophotometrically at 660 nm. Percent inhibition of denaturation by the sample was calculated using the given equation:

Inhibition (%) =
$$\frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Where, Abs $_{control} = O$. D of BSA without the sample, Abs $_{sample} = O$. D of BSA with the sample.

In-vitro Anti-haemolytic Assay: The procedure of anti-hemolytic assay was carried out according to the method described by Henkelman *et al.*, ²⁸ using hydro-alcoholic sample extracts. Blood from healthy volunteers was collected into centrifuge tubes containing 3.2% of sodium citrate which was used as an anticoagulant.

It was then centrifuged at 1000 rpm for 10 min at 4°C. Plasma and the white buffy layer were removed carefully. The erythrocytes were washed thrice for 5 min with $1 \times PBS$ pH 7.4. 100 µl of test samples were mixed with 50 µl of 10 dilution erythrocytes (100 µl erythrocytes suspension: 900 μ l of 1× PBS). 1× PBS and 1% SLS were used as negative and positive controls respectively. The incubation was carried out for 60 min at 37 °C in a water bath. 850 µl of 1×PBS was added to each vial to make up the volume to 1 ml. The reaction mixture was centrifuged at 300 rpm for 3 min. The concentration of the hemoglobin in the supernatant was measured spectrophotometrically at 540 nm. The percentage inhibition of hemolysis was calculated using the equation:

% Haemolysis inhibition= 100 - [sample / control] \times 100

In-vitro HMG CoA Reductase Enzyme Activity Assay: The HMG CoA reductase enzyme activity was determined according to the procedure described by Venugopala Rao and Ramakrishnan ²⁹. The hydroalcoholic extracts were found to have a high amount of phytochemicals that were determined previously and hence were chosen to determine the HMG CoA reductase activity. The procedure was carried out using chicken liver. About 100 mg of chicken liver tissue was treated with 1 ml of 1g/L arsenate solution and homogenized well in a mortar and pestle. 0.5 ml of this tissue homogenate was mixed well with the same volume of 50 ml/L diluted perchloric acid and centrifuged at 3200 rpm for 10 min at 4 °C after an incubation period of 5 min. The mixture was filtered, and 0.5 ml volume of it was treated with 0.5 ml of freshly prepared hydroxylammonium chloride pH 5.5 of 2 mol/L concentration for the determination of HMG CoA and another reaction mixture with pH 2.1 for mevalonate.

After an incubation period of 5 min, 0.2 ml of the hydroalcoholic sample extracts were added to the respective tubes and was incubated for 20 min. After the incubation, 1.5 ml of ferric chloride reagent was added to the solutions for both HMG CoA and mevalonate and mixed well. The absorbance was taken after incubating the test tubes for 10 minutes at 540 nm spectrophotometrically. 1g/L of arsenate solution treated similarly was used as a blank. The ratio of HMG CoA to mevalonate was taken as an index to the enzyme activity. A

higher ratio indicates a lesser enzyme activity and *vice versa*. The samples showing lower enzyme activity were considered for further tests.

Solvent-solvent Partition: The process was performed according to the protocol of Rahman et al., ³⁰ with a few modifications. Three different solvents- hexane, ethyl acetate, and chloroform were used for the process. Equal volumes of hydroalcoholic extract of Rosa centifolia and hexane were mixed vigorously for 5 min and allowed to stand till the organic phase separated and was collected. The sample treated with the solvent was centrifuged at high speed for 8 min to enable the residual solvent to separate and the fraction of interest was collected. The sample was treated similarly with ethyl-acetate and chloroform, and the separating organic phases were collected and subjected to HMG CoA reductase enzyme activity assay. The extracts showing minimum enzyme activity was selected for further study.

Column Chromatography: The *Rosa centifolia* hexane extract was subjected to column chromatography for partial purification of the sample. Silica gel was used as the stationary phase and 70% ethyl alcohol was used as the mobile phase. 1 ml of the sample was injected into the column, and the flow rate was adjusted to 0.2 mL per min. 1 ml of the elution was collected in vials, and the optical density was determined at 254 nm, 280 nm, and 418 nm to determine different compounds present in the sample.

RESULTS AND DISCUSSION:

Comparison of Anti-inflammatory Effects of the Sample Extracts: Protein denaturation is one of the well-known causes of inflammation. Proteins upon denaturation lose their secondary and tertiary structure on account of external stress such as varied acid, base or salt concentration, heat, *etc*. Protein denaturation in eukaryotes is carried out by a process called ubiquitination mediated by the 76 amino acid long polypeptide ubiquitin ³¹.

The comparative anti-inflammatory effects of the sample extracts were calculated and plotted as seen in **Fig. 1**. It was observed that the hydroalcoholic extract of *Coriandrum sativum* showed the highest value for the inhibition of protein denaturation (95.83%), thus explaining the potent anti-inflammatory activity of *Coriandrum sativum*,

followed by that of *R. centifolia* and *C. dactylon*. However, the less anti-inflammatory effect was observed in the aqueous extract of *Cynodon dactylon* (25%). Aspirin that was used as the standard drug for the anti-inflammatory action showed 64.18% protein denaturation inhibition at 0.1 mg/mL concentration and 68.56% inhibition at concentration 1.0 mg/mL against control.

Non-steroidal anti-inflammatory drugs manage inflammation by the prevention of formation of prostaglandins *via* restricting the activity of cyclooxygenase enzyme (COX) ^{32, 33}. Aspirin is an example of a non-steroidal anti-inflammatory drug. Though NSAIDs have been attested of their antipyretic, analgesic, and anti-inflammatory actions they are also known to have side effects such as peptic ulcerations, intra-mucosal bleeding, *etc.* ³⁴ This raises the need of use of a natural



FIG. 1: ANTI-INFLAMMATORY EFFECTS OF THE SAMPLE EXTRACTS. Each experiment was performed in triplicates and the results are expressed as mean percentage protein inhibition \pm SD.

The results of the anti-hemolytic assay conducted on the hydroalcoholic sample extracts as depicted in **Fig. 2** implied that the samples have appreciable anti-hemolytic activity with *Rosa centifolia* sample being the most potent, followed by *Cynodon dactylon* and *Coriandrum sativum*. This indicates that the various phytochemicals present in the samples quenched the H_2O_2 before it could attack the erythrocytes and thus prevented hemolysis ³⁶.

Comparative Study of the Inhibition of HMG CoA Reductase by the Hydroalcoholic Sample Extracts: The enzyme assay showed that the drug Atorvastatin (1 mg/mL) increased the HMG CoA/ mevalonate ratios (2.109) indicating reduced HMG CoA reductase activity. The HMG Co A Reduction inhibition by the drug Atorvastatin is by the inhibition of MYC phosphorylation and its compound as a drug that can have antiinflammatory property and not have any side effects. *Coriandrum sativum* is reported to have been used in the treatment of bed cold, nausea, stomach disorders and also used as carminative and diuretics. Coriander plant products are used in every household. Hence incorporation of coriander stems as a part of treatments to prevent inflammation due to the action of other drugs can be helpful as also initial researches suggest the use of coriander for treatment as well as for the prevention of many chronic diseases.

Comparison of the anti-hemolytic activity of the hydroalcoholic sample extracts: Most plant samples contain substances that have a hemolytic effect on human erythrocytes which results in other adverse effects. Hence, the need arises to analyze the sample for its hemolytic activity ³⁵.



FIG. 2: ANTI HAEMOLYTIC ACTIVITY OF THE SAMPLE EXTRACTS. The experiment was carried out in triplicates and the results are expressed as mean percentage haemolysis inhibition \pm SD.

activation. This result of a study conducted establishes that the enzyme HMG CoA reductase also acts as a critical regulator of MYC activator and is thus effective against the MYC-induced cancers ³⁷. The control with the lowest HMG CoA/ mevalonate ratio (0.70) indicates the HMG CoA reductase activity to be high implying the uninhibited enzyme and high biosynthesized cholesterol levels.

The result of the study plotted in **Fig. 3** revealed that the enzyme HMG CoA reductase is inhibited significantly by all the three hydroalcoholic sample extracts with *Rosa centifolia* having the maximum inhibitory activity (ratio=1.4117) in comparison with *Coriandrum sativum* (ratio=1.099) and *Cynodon dactylon* (ratio=1.0684) extracts. The HMG CoA reductase inhibition might be due to the

presence of sterols or isoprenoids in the plants such as the gibberellic acid sprayed on peas³⁸.



FIG. 3: *IN-VITRO* HMG COA REDUCTASE ACTIVITY IN THE PRESENCE IF HYDROALCOHOLIC EXTRACTS. The experiment was performed in triplicates and the results expressed in mean ratios \pm SD.

Comparison of HMG CoA Reductase Enzyme Activity in Different Solvent Fractions of *Rosa centifolia* **Hydroalcoholic Extract:** The solventsolvent extraction was carried out using three solvents: hexane, ethyl acetate, and chloroform with the hydroalcoholic extract of *Rosa centifolia*. The enzyme activity assay was performed and plotted as seen in **Table 1**. Out of the three fractions, the hexane fraction showed the highest HMG CoA/mevalonate ratio. It can be deduced from this result that the potential inhibitor of the HMG CoA reductase enzyme is present in the hexane fraction. The drug Atorvastatin at 1 mg/mL concentration showed significant inhibition of the enzyme HMG CoA reductase (ratio= 2.01).

TABLE 1: HMG COA REDUCTASE ENZYMEACTIVITY IN DIFFERENT SOLVENT FRACTIONS

Sample	Ratio
Control	0.5523
Drug	2.01
Hexane fraction	1.4956
Ethyl acetate fraction	0.7273
Chloroform fraction	0.7156

The experiment was performed in triplicates, and the values are represented as the mean of ratios.

Partial Purification and Spectrophotometric Analysis of Partially Purified *Rosa centifolia* **Hexane Fraction:** The sample hexane fraction subjected to column chromatography was eluted using 70% ethanol as the eluent and silica gel as the matrix. Flavonoids, proteins, and alkaloids are reported to be determined at 254 nm, 280 nm and 418 nm respectively. The elutions were screened only for the presence of proteins, total alkaloids and flavonoids at their respective wavelengths spectrophotometrically. It was observed that flavonoids were found in abundance at the 10^{th} elution. Total alkaloids were found to be eluted in increasing amounts after the 12^{th} elution.



FIG. 4: CHROMATOGRAM OF HEXANE FRACTION OF ROSA CENTIFOLIA

CONCLUSION: This study focussed on replacing the synthetic statins with the natural, plant products to reduce or avert the toxicities of the synthetic ones. The outcome of the work is that the extracts of *Rosa centifolia* have potent anti-hyper-cholesterolemic activity.

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CONFLICT OF INTEREST: None

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