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An in-vitro assessment of anti-inflammatory, antioxidant, and anti-hyperglycemic activities of traditional edible plants - *Murraya koenigii, Mentha spicata,* and *Coriandrum sativum*

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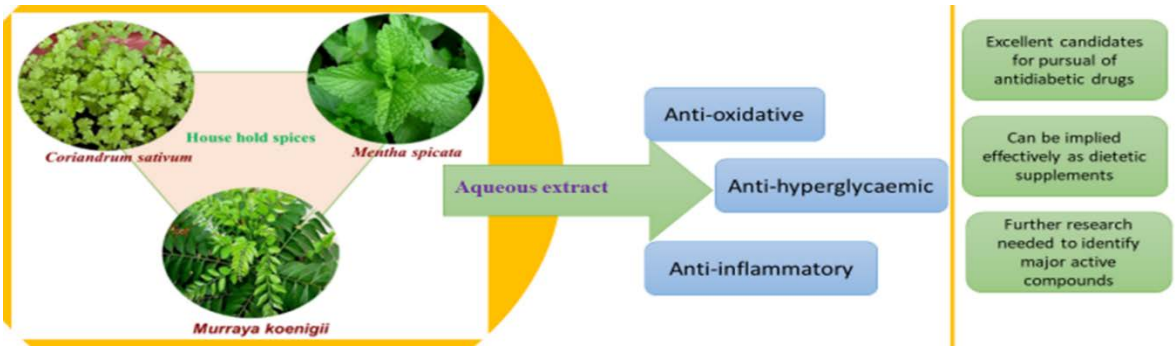
ABSTRACT



Since ancient times, Indian cuisines have included several spices and herbs due to their distinct aroma. The role of spices is to increase palatability and as flavoring, coloring, and preservative agents. Besides cooking, spices are also known for their medicinal properties, hence are a part of many pharmaceutical and medicinal industries. Although the spices are used for medicinal purposes by folks as a homemade remedy, the lack of scientific evidence makes their acceptance in modern medicine difficult. The advancement of side effects and ineffectiveness of synthetic medicines lead to a renewed interest in spices and herbs. Here, we evaluate three broadly used ingredients in Indian cuisines, i.e., *Murraya koenigii, Mentha spicata,* and *Coriandrum sativum,* for their biological constituents and role as anti-inflammatory antioxidants anti-hyperglycemic agents. With the help of various biochemical tests, we found that *Mentha spicata* has the best anti-inflammatory activity along with good antioxidant and anti-hyperglycemic activity, followed by *Coriandrum sativum* and *Murraya koenigii.* This study paves the way for finding new clinically efficient compounds against chronic inflammation together with Diabetes Mellitus.

Keywords: Coriandrum sativum, anti-inflammatory activity, antioxidant activity, anti-hyperglycemic activity

INTRODUCTION

Medicinal properties of herbs and spices have been recognized in India since the dawn of time. These edible plants are traditionally involved in Indian foods for their distinct taste and aroma and improve health and prevent certain diseases. Besides, practical advancement in medical science has brought the need for scientific evidence to qualify legal approval for comprehensive utilization of a drug in public. In this regard, we have chosen *Murraya koenigii*, *Mentha spicata*, and *Coriandrum sativum*, also known as curry, mint, and coriander, respectively, to analyze their phytochemical properties and efficacy as potent anti-inflammatory, antioxidant and antidiabetic compounds. All three plants are well-known herbs, ubiquitous in nature, and extensively used in garnishing and salad preparation. The green leaves of *M. koenigii*, *M. spicata*, and *C. sativum* are stated to be eaten raw for curing dysentery, and the infusion of the washed leaves stops vomiting.[1–4] *M. koenigii* (curry leaves), a member of to Rutaceae family, has Vitamin A, Vitamin B, Vitamin C, Vitamin B2, Calcium, and iron in plenty. Its nutritional value benefits both the young and the old alike. Women who suffer from calcium deficiency, osteoporosis, etc., can find an ideal

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natural calcium supplement in curry leaves. The curry leaves, ground to a fine paste and mixed with buttermilk, can also be taken on an empty stomach with beneficial results in stomach upsets, hence commonly used as a laxative. Curry leaves paste also used to treat persistent boils.[5,6]

Similarly, *M. spicata* is the third-largest liked flavour worldwide and one of the foremost spices extensively used for flavourings in food, cosmetics, and pharmaceuticals worldwide. It belongs to a small genus of aromatic perennial herbs, Lamiaceae, distributed mainly in the world's temperate regions. These plants are found to be rich in phenolics which positively influence human health by inhibiting the oxidation of low-density lipoproteins and the growth of bacteria, viruses, fungi, and by stimulating the growth of beneficial bacteria and activating or inhibiting enzymes that bind a specific receptor. Commercially, *Mentha* is also an essential oil-bearing plant with vast usage of spearmint, peppermint, and corn mint essence as a repellent, insecticidal, antibacterial, and antifungal agents.[2] The green leaves of *Coriandrum sativum* are consumed as fresh herbs, in salads, and as garnishes due to their attractive green color and aroma. Traditionally, coriander has been used to treat gastrointestinal disorders such as anorexia, dyspepsia, flatulence, diarrhoea, pain, and vomiting.[3] Different parts of the coriander plant have been reported for multiple health functions and biological activities, including antifungal, antibacterial, antioxidant, and sprout suppressant activities.[7] Despite these findings and traditional uses, these herbs are less trusted in modern medicine and require further research to proceed in the right direction.[8]

Diabetes mellitus is one of the major global health problems characterized by chronic hyperglycaemia with abnormal carbohydrate, fat, and protein metabolism due to defects in insulin secretion, insulin action, or both demanding preventive and new therapeutic interventions.[9,10] The hyperglycaemia may also have consequences like excessive non-enzymatic glycation of proteins and formation of advanced glycation end products (AGE). The glycation modifications can further deteriorate the diabetes pathogenesis by giving rise to nephropathy, cataracts, vasculopathy, and atherosclerosis.[11] The synthetic drugs like acarbose and miglitol show strong inhibition of important glycosidic enzymes, α-amylase and α-glucosidase; however, they may result in abdominal distention, flatulence, vomiting, and diarrhea.[12] Currently, there is a need for safe, effective, and less costly dietary supplements rather than promoting medicines, and hence investigating medicinal plants for dietary supplements is an exciting research area as it is natural and native to human beings and confers less side-effects to the body.

Inflammation is considered a complex biological retaliation of tissues to harmful stimuli.[13] The primary signs of inflammation include rashes, swelling, and pain.[14] Prolonged or chronic inflammation causes a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.[15] It is also associated with increased protein denaturation, vascular permeability, and membrane alteration. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage inflammatory conditions. However, these drugs have several adverse side effects, especially gastric irritation, leading to the formation of gastric ulcers.[16] Therefore, the search for natural sources and phytochemicals with anti-inflammatory activity has dramatically increased in recent years.[17]

The present study explores several medicinal benefits of the plants mentioned earlier to use as dietary supplements, which may lessen the medicinal burden in society. We aim to evaluate the anti-hyperglycemic, anti-inflammatory, and antioxidative effects of the aqueous extract of *M. koenigii, M. spicata,* and *C. sativum* using in vitro assays. Qualitative and quantitative tests have been performed to identify the major bioactive components in these plants. An antioxidant can be broadly defined as any substance that retard or inhibits oxidative damage of a target molecule caused by free radical, reactive oxygen species (ROS), and reactive nitrogen species (RNS).[18] The primary role of an antioxidant is to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols, and flavonoids scavenge free radicals such as peroxide, hydroperoxide, or lipid peroxyl, thus inhibiting the oxidative mechanisms that lead to degenerative diseases.[19] Hence, the plant extracts were assayed for their anti-oxidant activity, which can be considered as preliminary test for anti-hyperglycemic activity. This study cements the medicinal value of these plants and unlocks the prospect of finding new clinically efficient compounds, and will open the way for a further inquisition into the field of pharmacology and food chemistry for better drug discovery.

MATERIALS AND METHODS

Sample and Material collection

All plant samples were bought from a local market. The plants were verified by Department of Botany, University of Delhi. Their leaves were separated and dried for one day. After obtaining the powder forms of all three plants, their aqueous extracts were prepared, and further experiments were performed. All the solvents and chemicals used in biochemical tests were bought from Merck Millipore. 2,2-Diphenyl-1-Picrylhydrazyl (DPP extrapure, CAS no. 29128), Alpha-amylase porcine pancreas, 10U/mg (Cat no. 28588), Alpha-glycosidase ex. Yeast, 100Umg (Cat no. 75551), Trypsin 1:250 ex. Bovine Pancreas (Cat no. 74055), Bovine serum albumin (BSA, 98%, Cat no. 83803), and Acarbose 95% (Cat no. 65457) were bought from SRL chemicals, India. Anti-inflammatory drugs (NSAIDs), i.e., Diclofenac and Paracetamol, were bought from the local pharmacist.

Extract preparation

All extracts were made in distilled water to check their activity in a condition similar to body fluid as up to 90% of the human body weight comes from water, up to 60% of the human adult body is water (Mitchell et al.) As the above-mentioned plants are mostly used to prepare sauces, water was preferred over other solvents. 20 grams of the powdered leaves of each plant were weighed and dissolved in 200 ml distilled water (1:10) separately. The plant leaves were macerated with the help of a magnetic stirrer for 24 hours at 37^0C. The extracts were prepared by

vacuum filtration and rota evaporation. The following formula calculated the percentage yield:

% Yield = Extract / Powder form taken *100

50mg/ml stocks were prepared for all extracts in distilled water for further evaluation.

Phytochemical Analysis

The qualitative and quantitative assays were performed to note the constituents of aqueous extracts of the above-mentioned plants. The presence of different crude phytochemicals was checked using various biochemical tests explicitly designed for Alkaloids, phenol, flavonoid, saponin, steroids, tannin, and terpenoids. The screening was done as per the standard method(s) explained in Table 1.

Table 1: Qualitative Analysis Test Methods[20–22]

Phytoconstituent	Chemical Test	Positive Result Observation
Tannins	2 ml of the extracts dissolved in 90% ethanol, boiled for 5 min, after 3 min incubation at room temperature, few drops of 5% $FeCl_3$ was added.	greenish to black colour formation
Steroids	few drops of acetic anhydride and concentrated H_2SO_4 were added to a 2ml extract dissolved in chloroform.	Green to greenish-blue coloration
Flavonoids	1g powdered sample was heated with 10 ml ethyl acetate over a steam bath (40–50°C) for 5 min. The filtrate was treated with 1ml dilute ammonia.	yellow coloration
Saponins	2g of the extract was mixed in 20 ml of distilled water in a hot water bath and filtered the solution. The filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable, persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously.	Emulsion formation
Phenols	2ml extracts were taken and an equal amount of 5% $FeCl_3$ and 90% ethanol solution were added to the extract.	Deep bluish-green solution
Cardiac Glycosides	1% $FeCl_3$ solution was prepared using 5% glacial acetic acid. 3ml extract was mixed in equal amounts of $FeCl_3$ solution. A few drops of concentrated H2SO4 were added.	Deep red color
Alkaloids	Extracts were dissolved with 5ml methanol and 5ml of 2N HCl and filtered. The filtrate was treated with Mayer's (Potassium Mercuric Chloride solution) and Wagner's (aqueous solution of Iodine and Potassium Iodide) reagents.	Creamy white precipitate formation (Mayer's test), red precipitate (Wagner's test)
Terpenoids	The extract was mixed in 2ml of chloroform. Then 3ml of conc. H_2SO_4 was added to form a layer.	reddish-brown coloration of the interface

Based on the presence of phytoconstituents noted in the qualitative analysis, total phenol, total tannin, and total flavonoid were determined. The methods followed for quantitative assessment are as follow.

Total Phenol: A slightly modified Folin and Ciocalteu method was adopted for phenols. Briefly, 0.5mL of the plant extract (100 µg/mL) was mixed with 2mL of 10% Folin-Ciocalteu reagent solution with 4mL of Na_2CO_3 solution (7.5%w/v) as a neutralizer. The reaction mixture was incubated at room temperature for 30 min at 37℃ in the incubator shaker. The absorbance of the resulting blue color was measured at 765nm. Gallic acid was used as a reference standard for plotting the calibration curve. The total phenol content was expressed as microgram Gallic acid equivalent (GAE) per milligram of dry extract.[23]

Total tannin: The Vanillin-HCl method determined the tannin content with slight modifications. Briefly, 400µL of extract (100µg/ml) is added to 3mL of 4% vanillin solution in methanol and 1.5mL of concentrated HCl. After incubation for 10 minutes at room temperature, absorbance was checked at 560nm. The serial stock of catechin (20, 40, 60... 100µg/ml) in methanol was used as a reference compound to obtain a calibration curve. The total tannin was expressed as microgram Catechin Equivalent (CE) per milligram of extract.[24]

Total flavonoid: The flavonoids were evaluated by Aluminum Chloride ($AlCl_3$) Colorimetric Assay. In this assay, extract solutions in water (0.5 ml), were incubated with 10% $AlCl_3$ (0.1 ml) and 1M CH_3COOK (0.1 ml). The volume was made up to 5ml by adding distilled water (4.3 ml). The reaction tubes were incubated at room temperature for 30 minutes, and the absorbance was measured at 415nm. Quercetin was used to make the standard calibration curve. The total flavonoid content in the extracts was calculated in triplicate, and the results were averaged. The total flavonoid was expressed as microgram Quercetin Equivalent (QE) per milligram of extract.[25] All tests were performed in triplicates.

Anti-inflammatory Activity

The anti-inflammatory activity was checked on plant extracts by performing three different assays viz, Membrane Stabilization Assay, Protease Inhibition Assay, and Protein denaturation assay.

Membrane Stabilization/Cell lysis Inhibition Assay

Methods of Saket et al. (2010) was followed with minor modifications. Fresh whole human blood* (10 ml) was collected and mixed with an equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm for 10 minutes. Pellets obtained were washed three times with isosaline (0.85%, pH 7.2). 10% v/v suspension of blood was made with isosaline. The stock erythrocyte (RBC) suspension (0.50 ml) was mixed with 5ml of hypotonic solution (50mM NaCl in 10mM sodium phosphate-buffered saline; pH=7.4) containing the extracts and standard NSAID drug, Diclofenac sodium with concentrations 10-100 µg/ml. The control sample consisted of 0.5 ml of RBC mixed with hypotonic buffered saline solution alone. The mixture was centrifuged for 10 min at 3000 g after 10 minutes of incubation at room temperature. The

absorbance of the supernatant was measured at 560nm.[26] The percentage inhibition of hemolysis or membrane stabilization was calculated according to the formula below

% Haemolysis Inhibition = 100 - (Abs test/Abs positive control) × 100

Where Abs positive control represents the absorbance of positive control (RBC without extracts/standard); Abs test represents the absorbance of test samples (RBC incubated with extracts/standard)

* blood was voluntarily provided by the authors Miss. Zohra Hashmi and Miss. Dhoopchhaya Sarkar for the experiment. Both individuals were healthy and under no medication.

Anti-Protease Assay

During infection, pathogens invade tissue by using exotoxins, in which several types of protease play an important role by activating several signaling pathways. The presence of microbial proteases in blood serum acts as a hallmark of infection. Hence, protease inhibitors hold significance in treating several diseases and inflammatory responses. However, few protease inhibitors are available for treating bacterial infections and diseases. *Mentha spicata*, *Coriandrum sativum*, *and Murraya koenigii* extracts were checked for protease inhibitory response by pre-incubating Trypsin [SRL, India (2000 units/g)] with the extracts (10µg/ml to 100µg/ml) or standard drug (Paracetamol). 1mL aliquot of trypsin (0.5 mg/mL prepared in 0.1 M phosphate buffer pH 7) was pre-incubated with 1mL of a series of samples and standard drug, Paracetamol (10µg/ml to 100µg/ml) at 37° C for 15 min. 2mL of 1% bovine serum albumin (BSA) (SRL, India; prepared in 0.1 M phosphate buffer) was added and incubated at 37° C for 30 min. The reaction was terminated by adding 2.5mL of 0.44 M Trichloroacetic acid (TCA). The reaction mixture was centrifuged at 10,000rpm for 15 minutes, and the pellet was discarded. The absorbance of the supernatant was measured at 280nm in a UV–Visible spectrophotometer against appropriate blanks (0.1M phosphate buffer).[26] Appropriate blanks for the enzyme, inhibitor, and substrate were also included in the assay along with the test. Peptide fractions of casein due to trypsin action in the presence and absence of the inhibitor are TCA soluble. More the absorbance less is the inhibition. The below formula calculated percentage protease inhibition:-

% Protease Inhibition = 100 - (Abs test/Abs positive control) × 100

Where Abs positive control represents the absorbance of positive control (Protein with enzyme without extracts/standard); Abs test represents the absorbance of test samples (Protein and enzyme incubated with extracts/standard)

Heat-induced Protein Denaturation Inhibition Assay

The assay was performed as per the protocol mentioned by Leelaprakash, Mizushima, and Sakat with slight modification. The reaction mixture consisted of test extracts (10µg/ml to 100µg/ml) and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted to 7, using 1N HCl. The sample extracts were incubated at 37 ºC for 20 min and then heated to 51 ºC for 20 min. After cooling the samples, the turbidity was measured at 660nm.[26] Each assay was repeated at least thrice.

The following formula was used to calculate the percentage inhibition of protein denaturation:

% Inhibition = (Abs positive control –Abs test) X 100/ Abs positive control

Where Abs positive control represents the absorbance of positive control (protein denaturation in the absence of extracts); Abs test represents the absorbance of test samples (Protein denaturation in the presence of extracts).

Antioxidant Activity

There are specific biochemical assays that can detect the potential antioxidant activity. In most cases, antioxidant activity correlates with antidiabetic and anti-inflammatory activity. Hence, antioxidant activity was checked for all extracts by performing two assays; Phosphomolybdenum reduction assay, also known as reducing power assay, and DPPH radical scavenging assay, commonly known as Percentage Free radical scavenging assay.

Phosphomolybdenum Reduction Assay

The total antioxidant capacity of extracts was determined by incubating them with Mo (VI). Extracts with antioxidant capacity reduce Mo (VI) to Mo (V) in acidic pH, forming a green complex. 0.1ml of extracts with varying concentrations (10µg/ml to 100µg/ml) was incubated with 1ml of reagent solution (0.6M Sulfuric acid, 28mM Sodium phosphate, and 4mM Ammonium molybdate) at 95°C for 90 minutes. After the color development, the absorbance was noted at 695nm using a spectrophotometer. The test was repeated for a series of Ascorbic acid concentrations (10µg/ml to 100µg/ml), which was used as standard.[27] Appropriate negative and positive controls regarding all extract concentrations have been maintained to remove the error.

Free Radical Scavenging Assay

The DPPH assay was carried out as described by Souri et. al. in 2008.[28] The antioxidant activities of extracts were assessed by measuring their scavenging abilities to 2, 2-diphenyl-1-picrylhydrazyl (DPPH). In this method, DPPH, a stable free radical, was incubated with a series of extracts (10µg/ml to 100µg/ml). 0.1mM of DPPH solution was prepared in 100% methanol, from which 1ml of DPPH solution was mixed with 0.1ml of extract. After 30 minutes of incubation in the dark, the absorbance was noted at 517nm. Ascorbic acid (0.1mmol/ml to 1mmol/ml) was used as the standard for the assay. Appropriate negative controls regarding all extract concentrations have been maintained to remove the error. Each assay was performed three times. The percentage scavenging activity was calculated according to the formula of Yen & Pin-Der described in 1994.[29]

% Free Radical Scavenging = [(Abs positive control - Abs test) × 100] ÷ Abs positive control

Where Abs positive control represents the absorbance of positive control (DPPH solution without extracts/standard); Abs test represents the absorbance of test samples (DPPH incubated with extracts/standard).

Anti-hyperglycaemic Assay

Anti- hyperglycaemic activities of all extracts were assessed by performing two assays viz, α-amylase enzyme inhibition assay and α-glucosidase enzyme inhibition assay. Both enzymes are present in human beings; hence, their in-vitro inhibition directly links the extracts' anti-hyperglycemic activity.

α-Amylase Inhibition Assay

α-amylase Inhibition Assay was done per the protocol described by Tamil et. al. in 2010,[30] with slight modifications. Plant extracts (10µg/ml to 100µg/ml) were incubated first with the α-amylase enzyme (0.1U/mL) for 10 minutes at 37ºC and then with the starch solution (1% w/v) for 10 minutes. Acarbose, the common antidiabetic drug, was used as standard, and a similar concentration of acarbose (10µg/ml to 100µg/ml) was made (to compare the anti-hyperglycemic activity) and incubated with DNS reagent for 10 minutes at 37ºC in a water bath. The entire assay was performed in the 1X Phosphate Buffer Solution (1X PBS, pH 6.9). Absorbance was taken of standards and extracts at 540nm after diluting the content with distilled water. Enzyme Activity (EA) was calculated by plotting the absorbance value obtained of all samples and controls (positive control contained enzyme and starch solution while negative control contained only starch; both were mixed with DNS reagent.), and results were represented in % inhibition of the α-amylase enzyme, which was calculated as per the given formula:

% α-amylase Inhibition = 100 - (EA positive control - EA test)/EA positive control × 100

Where EA positive control means Enzyme Activity without inhibitor (Positive Control); EA test means Enzyme Activity with inhibitor (Extract or Standard drug).[30,31]

α-Glucosidase Inhibition Assay

The inhibition of *α*-glucosidase activity was determined using the method described by Dewi et. al. in 2007,[32] with slight modifications. In this assay, para-nitrophenol-α-glucoside (PNPG), as a substrate, was treated with an α-glucosidase enzyme, which is largely involved in various diseases, including Diabetes and HIV (viral fusion with T helper cells) in the presence of extracts. 0.1mL of α-glucosidase enzyme (0.1Unit/mL) was incubated with the 0.1ml of extracts with varied concentration (10µg/ml to 100µg/ml) for 5 minutes. After that, 0.1mL pNPG was added (3mM solution) to the reaction mixture tubes, and the total volume was made up to 1mL using 1X PBS, pH=7. The tubes were then incubated for 15 minutes at 37º C. In the end, 0.1mL of 0.2M Na_2CO_3 was added to the tubes for color development. The enzyme digests the substrate and releases p-nitrophenol phosphate (PNPP), a transparent substance indicating the enzyme activity. The enzyme activity inhibition was compared with the standard antidiabetic drug, acarbose. Three tubes with untreated enzyme and substrate were also incubated as a positive control. As the extracts impart their color and may add some erroneous reading, negative control for all extract samples has been maintained (extracts without substrate and enzyme), and reading was subtracted from the test sample readings. Results were represented in % inhibition of the α-glucosidase enzyme, which was calculated as per the given formula:

% α-glucosidase Inhibition= 100 - (EA positive control - EA test)/EA positive control × 100

Where EA positive control means Enzyme Activity without inhibitor (Positive Control); EA test means Enzyme Activity with inhibitor (Extract or Standard drug).[33]

Statistical analysis

All statistical analyses were performed using Graph Pad Prism 8 version 0.2) USA. One-way ANOVA analysis was used to evaluate differences between different standard extracts. Values of $p < 0.05$, $p < 0.001$ and $p < 0.0001$ was considered to be statistically significant. Means between treatments groups were compared for significance using Duncan's new multiple range post-test. All tests were repeated thrice, and the results were represented as Mean ± Standard Deviation (SD).

RESULTS AND DISCUSSION

Plant Extract Preparation & Phytochemical Profiling

Results for the total extract yields indicated that *Coriandrum sativum* gives the highest percentage yield (16.05%), followed by *Mentha spicata* (13.85%) and *Murraya koenigii* (10%). Qualitative analysis revealed the presence of tannins, flavonoids, and phenols in all three plants, i.e., *M. koenigii*, *M. spicata*, and *C. sativum* leaf extracts (Table 1) which might be responsible for their physiological properties and activities related to anti-inflammation, anti-hyperglycemic, and anti-oxidation). Hence their quantity was assayed in each plant. It was observed that *M. spicata* has the highest phenolic content with 1655.59 ± 1182.73 µg/mg GAE, followed by *C. sativum* 1287.37± 718.50 µg/mg GAE, and least in *M. koenigii* 1139.43 ± 366.51 µg/mg GAE. The total tannin content was found to be highest in the *M. koenigii* extract, (1021.5685 ± 62.80 µg/mg), followed by *C. sativum* and *M. spicata* (93.27± 6.27 µg/mg and 275.38± 12.56 µg/mg CE, respectively). Similarly, the flavonoid quantity was highest in *M. koenigii* (631 ± 205.57 µg/mg), while *M. spicata* and *C. sativum* showed comparatively lower tannin content with 109.08 ± 12.51 µg/mg and 27.71 ± 1.66 µg/mg QE, respectively (Figure 1). The extracts thus prepared were further analyzed for anti-hyperglycemic, antioxidant and anti-inflammatory activity.

Table 2: Qualitative analysis of the aqueous extracts of *Murraya koenigii* (Curry leaves), *Coriandrum sativum* (Coriander), *Mentha spicata* (Mint**).**

Constituents	*Murraya koenigii*	*Coriandrum* sativum	*Mentha spicata*
Tannins	++	+	+
Steroids	+	-	-
Flavonoids	++	+	+
Saponins	+	-	+
Phenols	+	+	+++
Alkaloids	-	-	-
Terpenoids	-	-	-

+; present +++; high intensity of presence –; absent

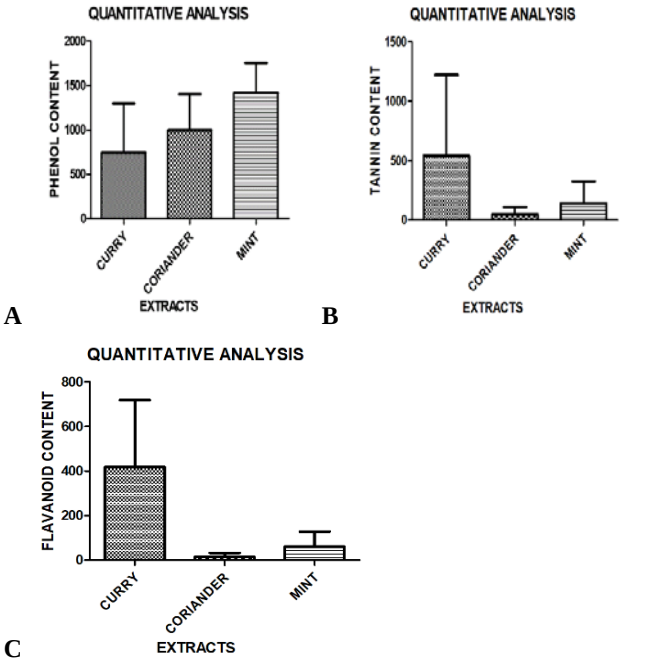

Figure 1: Bar graph representing quantitative analysis of phenol, tannins, and flavonoids in aqueous leaf extract measured in *Murraya koenigii* (Curry leaves), *Mentha spicata* (Mint), and *Coriandrum sativum* (Coriander). (A) total phenol content, (B) total tannin content, (C) total flavonoid content

ANTI-INFLAMMATORY ACTIVITY

In the present study, three herbal plants, i.e., *Mentha spicata*, *Murraya koenigii*, and *Coriandrum sativum*, were checked for their anti-inflammatory activity using cell lysis inhibition, anti-protease assay, and heat-induced protein denaturation assay.

Cell lysis Inhibition Assay

Among the three plant extracts evaluated for cell lysis inhibition activity, the crude extract of *M. spicata* was the most effective, followed by *M. koenigii* and *C. sativum*. *M. spicata* showed lowest IC_{50} value = 32.75 ± 2.04µg/ml followed by *M. koenigii* (IC_{50} = 249.9 ± 1.37µg/ml) and *C. sativum* (IC_{50} = 260.3 ± 2.3µg/ml). Nevertheless, all of them were found to have a greater in-vitro anti-inflammatory cell lysis inhibition activity than the standard NSAID, Diclofenac sodium (IC_{50} =276.60 ± 0.417µg/ml). The IC_{50} difference, when compared to the standard drug, was noted to be highly significant in the case of *M. spicata* (p value<0.0001) followed by *M. koenigii* (p value<0.05). No significant difference was observed with *C. sativum*. The above result suggests that the aqueous extracts of *M. spicata* and *M. koenigii* have more potency than the standard drug. Hence they may possess better bioactive components effective against chronic inflammation.

Anti-Proteinase Assay

The serine protease trypsin was used to evaluate the anti-protease activity of plant extracts and standard Paracetamol. All extracts showed higher anti-protease activity than Paracetamol, a standard antipyretic drug. *M.spicata* (IC_{50} = 8.696 ± 0.004µg/ml)) showed highest inhibition of protease enzyme, i.e., trypsin, followed by *C. sativum* (IC_{50} = 19.67 ± 0.004µg/ml). *M. koenigii* (IC_{50} = 24.93± 0.05µg/ml) showed lower inhibition than former two yet better than Paracetamol (IC_{50} = 54.73 ± 0.04µg/ml). All extracts except *M. koenigii* leaves were observed to follow a similar trend line to the Standard (Paracetamol). One-way ANOVA analysis revealed the higher significance of *M. spicata* and *C. sativum* (p-value < 0.0001) followed by *M. koenigii* (p-value < 0.001) (Figure 3).

Heat induced Protein denaturation Assay

Protein denaturation inhibition was observed in all extracts, in which *M. spicata* showed the highest inhibition (IC_{50} = 24.28± 0.002µg) followed by *C. sativum* (IC_{50} = 25.62 ± 0.03µg), and *M. koenigii* showed the lowest inhibition (IC_{50} = 54.29 ± 0.005µg). significant difference were between *M. spicata* and *M. koenigii* (p-value < 0.05), while the similar activity of *M. spicata* with that of *C. sativum* was reported with no significant difference (Fig 4). The ability of studied leaf extracts to prevent thermal protein denaturation may be responsible for their anti-inflammatory properties. However, the actual mechanism of this membrane stabilization is yet to be investigated further. It has been proposed that the extract might inhibit the release of the lysosomal constituents of neutrophils at the site of inflammation. Lysosomal constituents are bactericidal enzymes and proteinases that cause further tissue inflammation and damage.[34]

ANTIOXIDANT ACTIVITY

Antioxidant assay of the extracts was checked by performing two known biochemical assays – Reducing power Assay (also known as Phosphomolybdenum assay) and Free Radical Scavenging Assay (widely known as DPPH assay).

Reducing Power Assay

The reducing power of all extracts has been calculated by assessing the reduction of phosphomolybdenum (VI) to phosphomolybdenum (V) compared to Ascorbic acid as standard. Ascorbic acid exhibited the highest activity (IC_{50} value = 6.125± 0.05 µg/ml) followed by *M. spicata* extract, IC_{50} = 7.106±0.26 µg/ml, which showed a significant reduction of the substrate at a slightly higher concentration by developing deep blue color. Similar reducing powers were seen in the case of *C. sativum*, IC_{50} = 7.697±0.20 µg/ml while *M. koenigii* extract with IC_{50} = 8.675±0.94µg/ml showed significantly less reducing capacity and hence lower antioxidant activity as depicted in figure 5. The IC_{50} value of the extracts and standard showed their potential to reduce phosphomolybdenum and potential to be used as an alternative antioxidant in therapeutics.

Free Radical Scavenging Assay

A free radical scavenging assay was performed using DPPH assay as a confirmatory test.[35,36] Each extract's percentage free radical scavenging activity was compared with known standard, Ascorbic acid (IC_{50} value = 10.018± 0.04 µg/ml), used as a standard, exhibited the highest antioxidant activity. As expected, the trend of Ascorbic acid is closely followed by *M. spicata* extract with IC_{50} value = 15.85±0.26 µg/ml. In contrast to reducing capacity, *C. sativum* showed lower free radical scavenging property with higher IC_{50} value = 32.51±0.19 µg/ml than *M. koenigii* extract (IC_{50} value = 30.38±0.94 µg/ml).

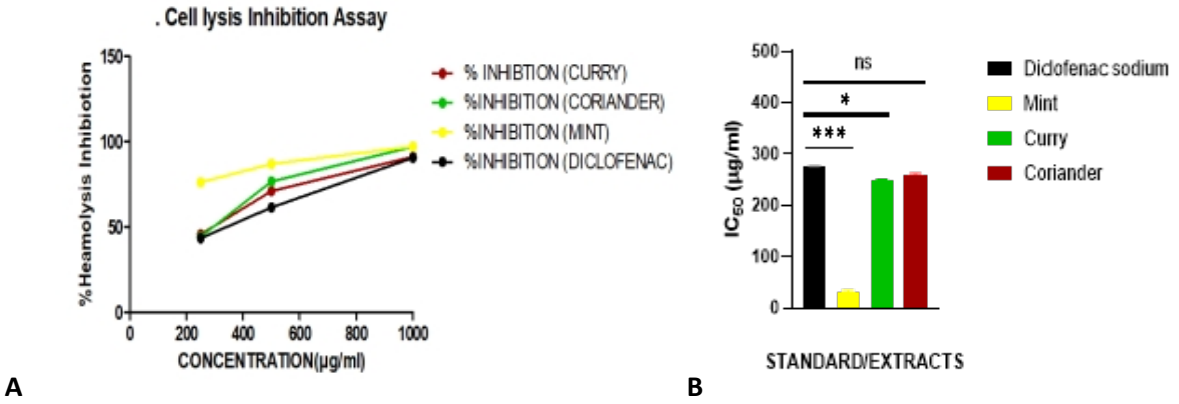

Figure 2: Anti-inflammatory activity of standard (Diclofenac sodium) and extracts (A) Line graph representing Percentage hemolysis inhibition of *M. spicata* (mint), *M. koenigii* (curry) and *C. sativum* (coriander) extracts, and Diclofenac sodium. (B) Bar graph depicting IC_{50} values of heamolysis inhibition effect of extracts/ standard (µg/ml). Data were represented as mean ± SD (N=3) *** p-value < 0.0001, *p-value < 0.05, ns = non-significant.

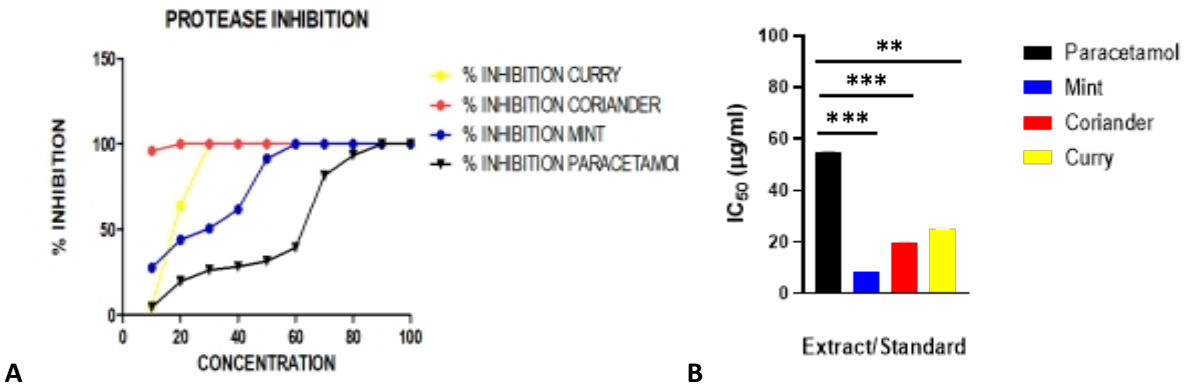

Figure 3: Protease inhibition assay for anti-inflammatory activity detection of standard (Diclofenac sodium) and extracts (A) Percentage inhibition of *M. spicata* (mint), *M. koenigii* (curry), and *C. sativum* (coriander) extracts and Paracetamol as standard. One unit of the X axis represents 20µg/ml extract concentration. 1unit of the Y axis is equivalent to 50% inhibition. (B IC_{50} values of protease inhibition effect of extracts/standard (µg/ml). Data were represented as mean ± SD (N=3) ** p-value < 0.001, *** p-value < 0.0001.

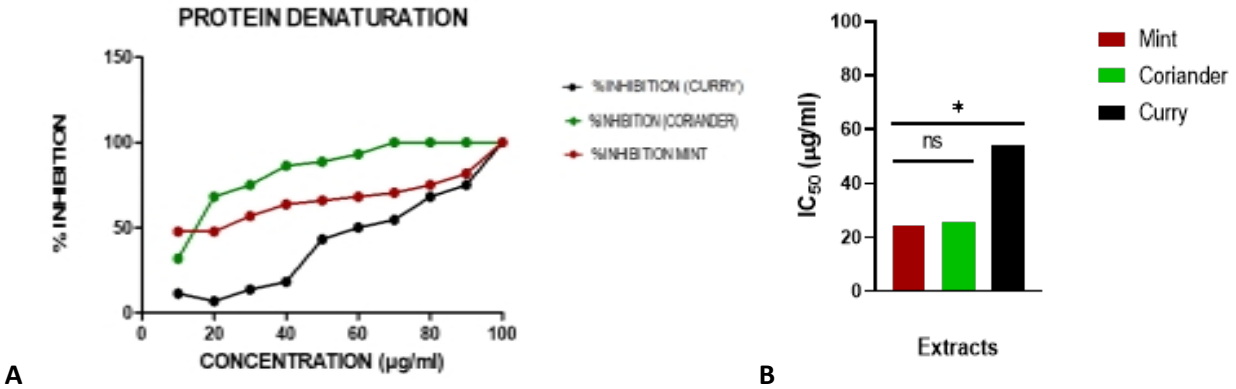

Figure 4: Protein denaturation inhibition assay for anti-inflammatory activity detection of extracts (A) % inhibition of *M. spicata* (mint), *M. koenigii* (curry), and *C. sativum* (coriander) extracts. (B) IC_{50} values of protein denaturation inhibition effect of extracts (µg/ml). Data were represented as mean ± SD (N=3) * p < 0.05, ns = non-significant.

Consistent with the result, one-way ANOVA analysis depicted that although ascorbic acid showed the best activity followed by *M. spicata*, the activity of *M. spicata* is not much lower than ascorbic acid ($p < 0.05$). At the same time, *C. sativum* and *M. koenigii* leaves showed much lower activity than *M. spicata* ($p < 0.0001$).

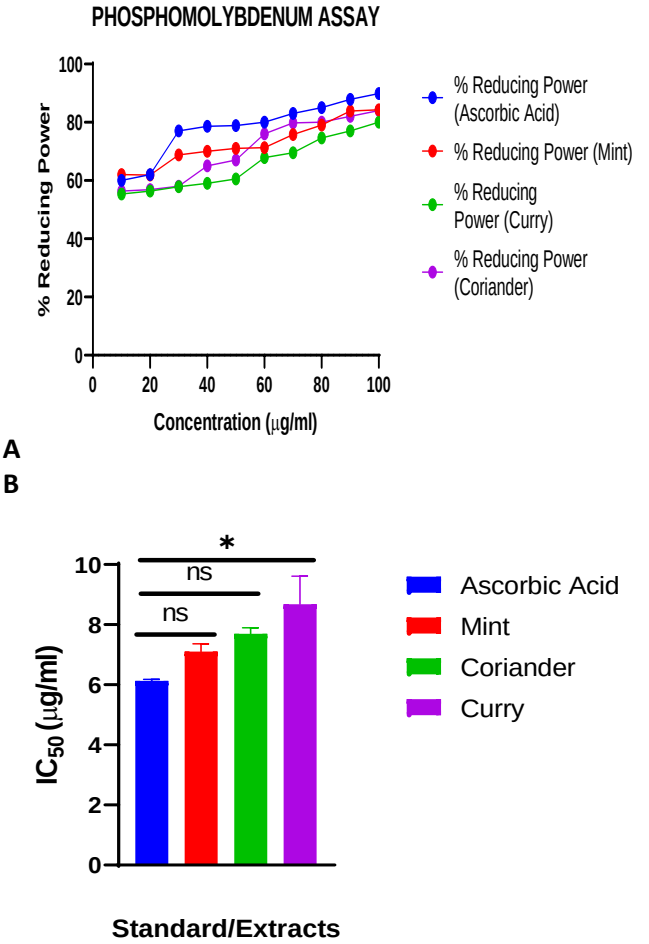

Figure 5: Antioxidant activity of standard (ascorbic acid) and extracts (A) % Reducing power of *M. spicata* (mint), *M. koenigii* (curry), and *C. sativum* (coriander) extracts and ascorbic acid as standard estimated by the reduction of Phosphate-Molybdenum (VI) to Phosphate-Molybdenum (V). (B) Bar graph depicting IC_{50} values of reducing power of the extracts/standard (µg/ml). Data were represented as mean ± SD (N=3) * $p < 0.05$, ns = non-significant.

ANTI-HYPERGLYCEMIC ACTIVITY

All extracts' anti-hyperglycemic activity was assessed by performing two assays, α-amylase enzyme inhibition assay, and α-glucosidase enzyme inhibition assay. Both enzymes are present in human beings; hence their in-vitro inhibition directly links the antidiabetic activity of the extracts.

Alpha-Amylase Inhibition Assay

Assessment of α-amylase activity revealed all extracts except *M. koenigii*, showed comparable enzyme inhibition activity as that of the standard antidiabetic drug, Acarbose (IC_{50} = 12.73 ± 1.17 µg/ml). *C. sativum* exhibited the best α-amylase inhibitory activity among the three plants assayed, having IC_{50} = 12.29 ± 0.19 µg/ml. *M. spicata* (IC_{50} value = 15.21 ± 0.26 µg/ml) was exhibited as almost equal anti-hyperglycemic property as standard acarbose. However, *M. koenigii* leaf extract showed minor activity with IC_{50} value = 31.63 ± 0.15 µg/ml. ANOVA test analysis also revealed that *C. sativum* have significantly higher activity ($p < 0.05$), whereas *M. spicata* and *M. koenigii* leave showed no significant difference from the standard acarbose.

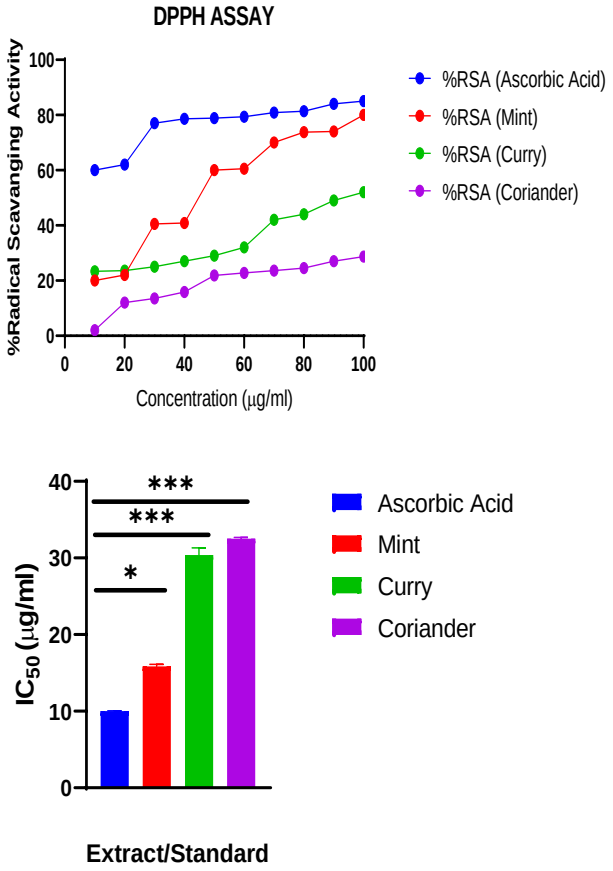

Figure 6: Antioxidant activity of standard (ascorbic acid) and extracts (A) % free radical scavenging power of *M. spicata* (mint), *M. koenigii* (curry), and *C. sativum* (coriander) extracts and ascorbic acid as standard). (B) IC_{50} values of extracts/standard's free radical scavenging effect of extracts/ standard (µg/ml). Data were represented as mean ± SD (N=3) * $p < 0.05$, *** $p < 0.0001$

Alpha-Glucosidase Inhibition Assay

Anti-hyperglycemic activity estimated by α-glucosidase enzyme inhibition assay depicted that *C. sativum* (IC_{50} = 0.370± 0.19 µg/ml) and *M. spicata* (IC_{50} = 0.388 ± 0.26 µg/ml) showed the best activity, even better than the standard antidiabetic drug, acarbose. However, *M. koenigii* showed lower inhibition of α-glucosidase enzyme than the standard with IC_{50} value = 6.555 ± 0.49 µg/ml. One-way ANOVA analysis also depicted that *C. sativum* and *M. spicata* showed significantly higher activity than the standard acarbose ($p < 0.001$). At the same time, *M. koenigii* leaves exhibited significantly lower activity than acarbose ($p < 0.0001$).

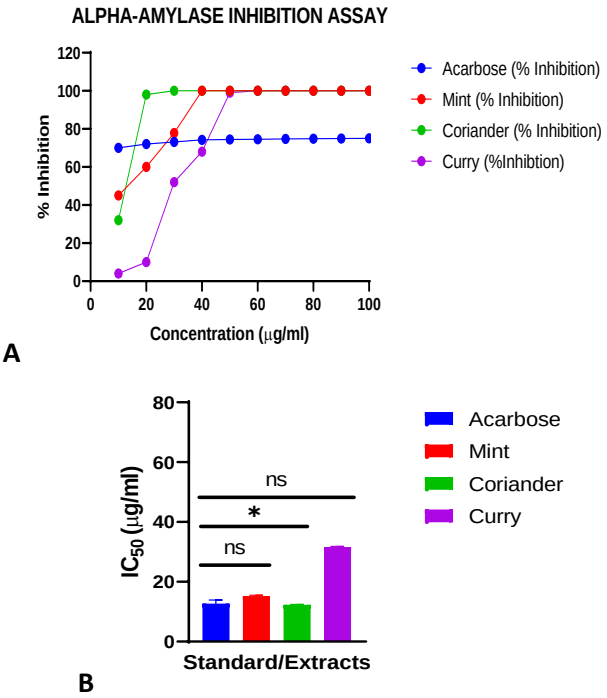

Figure 7: Anti-hyperglycemic activity of plant leaf extracts and standard (acarbose). (A) Line graph showing percent alpha-amylase inhibitory effect of *C. sativum* (coriander), *M. spicata* (mint), *M. koenigii* (curry) leaf extracts, and acarbose. One unit of the X-axis represents 20µg/ml of extract concentration. 1unit of the Y axis represents 50% of Enzyme Inhibition. (B) Bar graph representing IC_{50} values of the α-amylase inhibitory effect of extracts/ standard (µg/ml). Data were represented as mean ± SD (N=3) * $p < 0.05$, ns = non-significant.

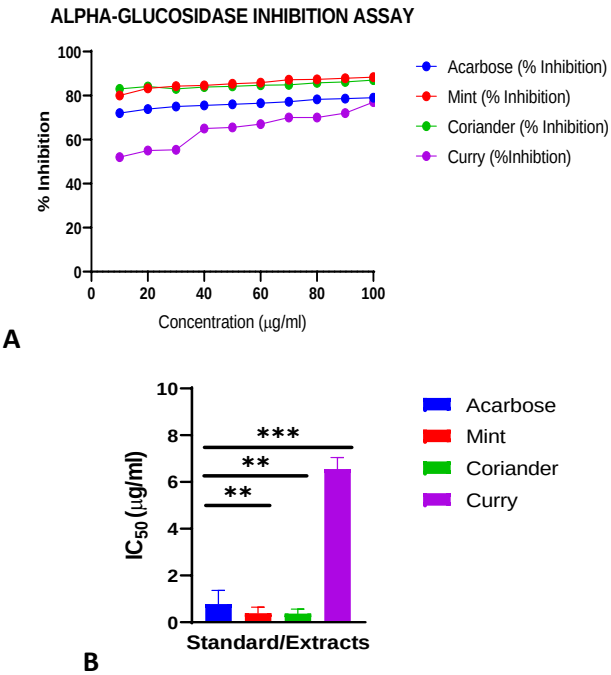

Figure 8: Graphs depicting Anti-hyperglycemic activity of plant leaf extracts and standard (acarbose). (A) Line graph comparing percent alpha-glucosidase inhibitory effect of *C. sativum* (coriander), *M. spicata* (mint), and *M. koenigii* (curry) leaf extracts with acarbose as standard. One unit of the X axis represents 20µg/ml of extract concentration. 1unit of the Y axis represents 20% of Enzyme Inhibition. (B) Bar graph representing IC_{50} values of α-glucosidase inhibitory effect of extracts/ standard (µg/ml). Data were represented as mean ± SD (N=3) ** ($p < 0.001$), *** ($p < 0.0001$).

CONCLUSION

India is well known for utilizing its ample plant resource as spices for food and medicinal purpose. Leaves of *Murraya koenigii* (curry), *Mentha spicata* (mint), and *Coriandrum sativum* (coriander) has been widely used as spices and sauces in small quantities for their distinct aroma, taste as well as preservative actions in Indian Cuisines. It is imperative that a healthy lifestyle prevents several diseases to the person. Recent studies suggest that diseases like Diabetes, Obesity, and Cardiovascular Disorder occur due to a sedentary lifestyle. We aimed at evaluating the use of *M. koenigii*, *M. spicata*, and *C. sativum* as an alternative for safe, effective, and less costly dietary supplements rather than promoting medicines in modern science. Extraction solvent plays an important role in the medicinal values of plants, several studies has demonstrated the anti-inflammatory and anti-oxidant activity of these herbs in ethanolic extract. Nevertheless, it is important to consider that a major proportion of human body is composed of water and evaluation of medicinal activity in similar condition is crucial for better assimilation. In this regard, we checked the in-vitro anti-inflammatory, antioxidant and anti-hyperglycaemic activity of the aqueous extract of the above plant leaves. Our study provides scientific evidence that *M. spicata*, *M. koenigii*, and *C. sativum* can be used as additives and dietary supplements as antidiabetic and antioxidant food formulations and as potential health-promoting ingredients. As reported, all extracts showed excellent in-vitro activity against inflammation. The crude extracts' activity was noted much better than the standard anti-inflammatory and antipyretic drug, Diclofenac sodium, and Paracetamol. This suggests the plants' potency to serve as a better treatment against chronic inflammation. *M. spicata* and *C. sativum* showed better antioxidant properties than *M. koenigii*, with *M. spicata* having comparable activity with standard ascorbic acid. *C. sativum* exhibited the best anti-hyperglycemic activity, followed by *M. spicata*. Although *M. spicata* showed comparable α- amylase inhibition to the standard antidiabetic drug, acarbose, α-glycosidase inhibition assay revealed better activity of *M. spicata* than acarbose. Nevertheless, these plant extracts showed excellent anti-inflammatory and good antioxidant and anti-hyperglycaemic properties, which can be further evaluated by in-vivo and ex-vivo studies on particular cell lines to assess further the potency and role of specific cells phytoconstituents.

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AUTHORS CONTRIBUTION

The study was conducted as Post-graduate dissertation training for the first two authors. The study was conceptualized and

supervised by Dr. Varsha Mehra, and assisted by Ms. Smita Mishra (Ph.D. Scholar). All authors equally contributed in writing the paper.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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