

# Thin Layer Chromatographic Profiling, Antioxidant and Antidiabetic Activity of Selected Medicinal Plants

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## ABSTRACT

Diabetes mellitus is a disorder whose frequency is speedily increasing everywhere on the planet. According to the World Health Organization (WHO), a United Nations Agency, polygenic diseases are going to be the seventh leading reason for death in 2030. Several healthful plants like *Citrullus lanatus*, *Trigonella foenum-graecum*, and *Murraya koenigii* are used for the treatment of diabetes disorders in the Indian medicine system and different ancient systems of the world. These three plant leaves were collected from Vadodara and Anand, Gujarat. Thin layer chromatography profiling of all three plant leaves confirmed the presence of various common classes of phytochemicals such as rutin, gallic acid, tannic acid, and quercetin in both cities. Thin layer chromatographic analysis of a particular leaf revealed various retention factor values in the range of 0.01 to 0.97. Also, *C. lanatus*, *T. foenum-graecum*, and *M. koenigii* plant leaves confirmed the antioxidant and antidiabetic activity but *C. lanatus* from Anand city was found to be the best plant. The study will help in the future to identify this plant for further research in industries and pharmaceutical companies.

**Keywords:** *Citrullus lanatus*, *Trigonella foenum-graecum*, *Murraya koenigii*, Thin layer chromatography, Antioxidant activity, Antidiabetic activity

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## INTRODUCTION

Diabetics have been documented since the Hindu scriptures. Diabetes is a metabolic form that ascends when the pancreas doesn't generate sufficient insulin or the physique does not use the insulin which is produced successfully. Insulin controls blood sugar levels. Elevated sugar level is a complication of uncontrolled diabetes, and it can cause significant harm to many organ functions, particularly the nervous system and blood flow, over time. Diabetes mellitus is an illness resulting from a variety of etiological factors that is characterized by high blood sugar as well as malfunctions through starch, fat, as well as carbohydrate metabolism due to diminished insulin sensitivity, insulin production, or even both (Ramachandran *et al.*, 2010). An antioxidant is an element that hinders other substances from oxidizing. They defend the critical components of cells by neutralizing the damaging consequences of radicals called free radicals, which are naturally produced by-products in the metabolic process of cells (Asmat *et al.*, 2016). Alpha-amylase is a member of the 1, 4-gluconohydrolase class and is an important objective enzyme in conventional diabetes management (Tundis *et al.*, 2010). Alpha-Glucosidase antagonists are employed to improve blood sugar control of high blood sugar through type two diabetes, especially postprandial hyperglycemia (Gong *et al.*, 2020).

Herbal plants are common in routine in our day-to-day life. Moreover, as a nutrient or a basis of food, the patient uses these herbs and a healthy person has easy access to raw consumption. It has minimum side effects and a low price. Diabetes and herbs have gotten a protracted relationship in the past. Therefore, plants are a potential provider of antidiabetic medication. These encompassed enhancing renewal or improvement of damaged pancreatic beta cells, and protects against additional injury, enhancing insulin synthesis and exudation from the beta-cells, decreasing glucose absorption from the gastrointestinal,

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cumulative insulin compassion of the tissues, keeping insulin imitating properties, and ever-changing the action of some enzymes concerned in glucose metabolism (Al-Snafi *et al.*, 2019).

*Citrullus lanatus* has conventionally been employed as a mild laxative, an emmenagogue in large doses, a vermifuge, a diuretic drug, and an energizer. Chronic renal infectious diseases, bedwetting, eye diseases, nephrotoxicity stones, alcoholism, diabetes, vomiting, oxidative stress, diarrhea, and gonorrhea are all treated with the seed (Erhirhie and Ekene, 2013). *Trigonella foenum-graecum* is rich in pectin, soluble and insoluble dietary, alkaloids, polyphenols, glycosides, as well as organic solvents. It serves a wide range of medical implementations, including antidiabetic, anticarcinogenic, hypocholesterolaemia, antioxidation, antimicrobial properties agent, hypoglycemia, gastrointestinal tonic, as well as anti-anorexia agent, among others (Aher *et al.*, 2016). *Murraya koenigii* plant's leaves are used as an antidiabetic, antioxidant, anticarcinogenic, a pain reliever, cure piles, and reduce body temperature, hunger, inflammatory processes, as well as itchiness (Balakrishnan *et al.*, 2020).

According to the Central Bureau of Health Intelligence's National Health Credentials from 2015, Gujarat has the strongest

diabetes prevalence in the nation. According to the health profile report, there are 1, 61, 578 diabetics in Gujarat, accounting for 20.5% of the overall 7, 87, 435 community checked. Diabetes covers approximately 4 crore people in India according to the International Diabetes Federation (Kinariwala *et al.*, 2020). Gujarat has 8 to 10% of the suspicious cases of diabetes. Almost 10% of scenarios are form one or juvenile diabetes, with the rest 90% being type two diabetes (Rana *et al.*, 2015).

*C. lanatus*, *T. foenum-graecum* and *M. koenigii* are a group of the families with viz., Cucurbitaceae, Fabaceae, and Rutaceae, respectively. Because Gujarat is a state where this plant is widely grown *C. lanatus*, *T. foenum-graecum* and *M. koenigii* plants were specifically chosen for the contemporary study conferring an analysis of the literature and ethnomedical information. The study of specific plant leaves is still lacking, and there hasn't been much research on their antidiabetic potential. As a result, the current study's goal is to inspect the leaves of these herbals using a clear scientific protocol.

## MATERIALS AND METHODS

### Collection and Processing of Plant Materials

*C. lanatus*, *T. foenum-graecum*, and *M. koenigii* leaves were collected uniformly from June-July of the year 2021 in Vadodara and Anand, Gujarat, India, and were fresh and healthy (Fig 1). Before shade-dried and made into a fine powder via a mixer grinder, leaves are washed to be free from dust. The powder is placed inside a clean flask by the solvent over time. Periodically enthused the content. A micelle is parted from the marc at the end of extraction through filtration. Powder of *C. lanatus*, *T. foenum-graecum*, and *M. koenigii* leaf was extracted through methanol, ethyl acetate, and petroleum ether, respectively (Abubakar and Haque, 2020).

### Thin Layer Chromatography (TLC)

Thin layer chromatography was completed on precoated silica gel thin layer chromatography plates grade F254 (E-Merck, Darmstadt, Germany) to regulate the sum of photoactive compounds present in the plant crude extract.

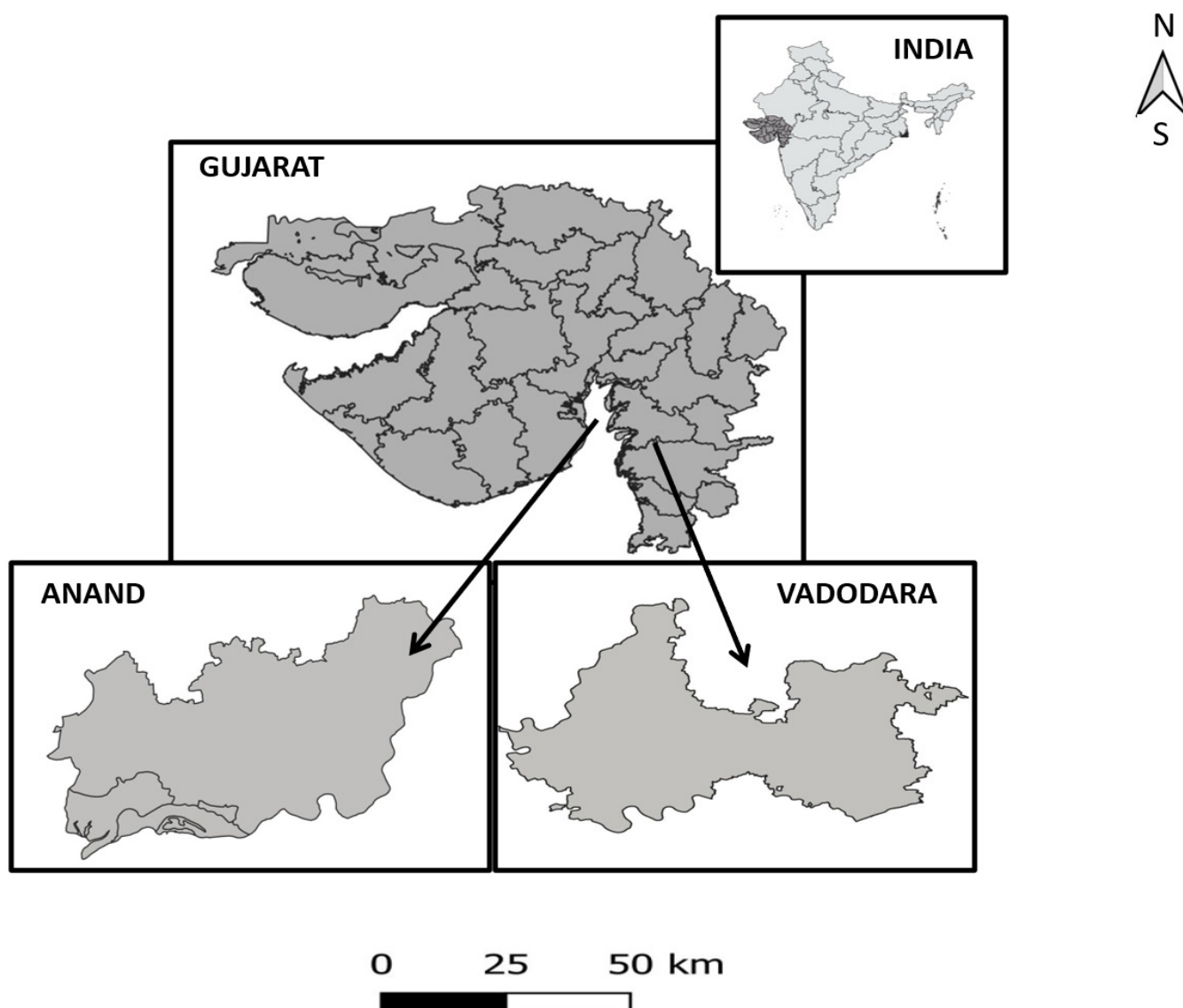


Fig 1: Study area showing Vadodara and Anand city , Gujarat, India

### Sample Application (spotting the thin layer chromatography plate)

One of the most basic practices for having applied TLC samples is with a glass capillary spotter. The spotting capillaries are highly small and simple to locate. Warm the capillary's middle section over a blue Bunsen burner flame. Once the central portion is hot sufficiently, it's going to become pliable, allowing you to quickly and smoothly separate the two ends. This will constrict the central portion into a thin string-like tube. After a few minutes of cooling, moderately break down the central string-like section into two-inch segments to deliver a good TLC capillary spotters. Fill the prepared capillary quickly with the organic sample solution

### Development of TLC Plates

Fill the chamber halfway to the development of the solvent. Place the TLC plate in the arranged chamber of development using tweezers, with the back-layer slanting in contradiction of the inside wall, and instantly cover the chamber through the lid. Here the solvent system of chloroform: methanol (9:1) is used. Keep in mind the starting line is higher than the solvent level. It must not be opened during development to maintain the atmosphere in the developing chamber. While possession, the chamber is closed, detect the front of the solvent through the side wall. When the thin layer chromatography plate has been immersed, the development process begins; when the front of the solvent has gotten an appropriate level, quickly remove the lid, remove the plate with tweezers, and mark the front of the solvent through a pencil. Make the plate dry until beginning the visualization process.

### Visualization

Simply placing the plate below an ultraviolet lamp allows the compounds to be seen with the naked eye. Only in very few cases is the sample dyed or colored and visible to the naked eye. It is known as non-destructive visualization. Because many substances absorb UV light, visualizing substances under ultraviolet light is much more common. TLC plates often include a fluorescent indicator that causes the thin layer chromatographic plate to glow green when exposed to ultraviolet light with a short wavelength of 254 nm. Substances that captivate ultraviolet light in the respective wavelength region will quench the green fluorescence, resulting in dark purple or bluish spots on the plate.

### Retention factor (Rf)

A quantity characterizes a participant compound's behavior in TLC. The retention factor is a decimal fraction. Each spot's retention factor value was assessed and compared to standard reference compounds. This formula is used to calculate the value of the retention factor (Bele and Khale, 2011).

$$\text{Retention factor} = \frac{\text{Distance traveled through the sample}}{\text{Distance traveled through the solvent}}$$

### In-vitro antioxidant activity

#### Free radical scavenging activity by diphenyl picrylhydrazyl (DPPH) free Radical

A 0.5 mg/mL concentration of leaf extract stock solution was prepared. In 4 mL of DPPH has been incorporated into 1-mL

of different concentrations of samples. The control has been made with a similar technique as the test only without the sample. Inside the absence of DPPH, ethanol has been substituted. For around 30 minutes, the response was allowed to take place inside the dark. The absorption spectrum was therefore assessed at 517 nm. As just a control, vitamin C was used. The sample concentration of sample required for just a 50% reduction through absorption intensity ( $IC_{50}$ ) has been determined by calculating (Rahman *et al.*, 2018).

### In-vitro Antidiabetic Activity

#### Inhibition of alpha-amylase enzyme assay

The plant extract as well as acarbose were diluted through Sodium buffer to make a 1mg/ml stock solution. 1 mL of 1% w/v starch solution, 1-mL of the alpha-amylase enzyme, and 2 mL of 0.1M sodium phosphate buffer were introduced to varying stock solution concentrations. A solution then was incubated for 1-hour at 37°C. The iodine-iodide indicator has been incorporated within an incubation. Color intensity was determined at 565 nm using a UV-visible spectrophotometrically. As just a control, 0.1M sodium-acetate buffer has been used. As a control, the reaction without extract was used. Acarbose was indeed a frequently used drug. Inhibition of enzyme action was intended through means of the following formulation:

$$\% \text{ Inhibition of enzyme activity} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample} \times 100}$$

A sample concentration needed for just a 50% reduction through absorbance ( $IC_{50}$ ) has been determined by calculating (Dineshkumar *et al.*, 2010).

#### Inhibition of alpha-glucosidase enzyme assay

0.1 mL alpha-glucosidase enzyme and 1-mL of 0.2M Tris buffer were added to the various concentrations of plant extract as well as acarbose. The combination was then incubated at 35°C for some time. The reaction then was stopped besides heating this for some time inside a boiling bath of water. The quantity of liberated glucose was evaluated using a UV-visible spectrophotometer at 540 nm using the glucose oxidation method. Acarbose was a commonly used drug. Except for the extract, the control was produced similarly. As a control, deionized water has been used. The formula was used to determine a percentage inhibition.

$$\% \text{ Inhibition of enzyme activity} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample} \times 100}$$

A sample concentration needed for just a 50% reduction through absorbance ( $IC_{50}$ ) has been determined by calculating (Anam *et al.*, 2009).

### Statistical Analysis

The data were denoted as mean  $\pm$  SD (standard deviation) and the data obtained from this study were subjected to an analysis of variance (ANOVA) and significance was resolute at  $p < 0.05$  (Adusei *et al.*, 2019).

## RESULTS AND DISCUSSION

Diabetes is a long-lasting metabolic condition that is turning into a risk to good health. The herbal principle has been claimed by traditional medicine to cure diabetes. This research

work aims to evaluate the antidiabetic potential of *C. lanatus*, *T. foenum-graecum*, and *M. koenigii* leaves from Vadodara and Anand, Gujarat.

### Thin Layer Chromatography

The purpose of the chromatography technique here in our experiment was to categorize compounds using the TLC technique. This method is advantageous in many ways, like it is easier to handle, cost-efficient, and quick. The amino acid identified in our experiments of *C. lanatus*, *T. foenum-graecum*, and *M. koenigii* leaves, which was identified based on retention factor comparing them with known compounds rutin, gallic acid, quercetin, and tannic acid is taken for the research. Dried ingredients are used frequently in phytochemical fields because they can be stored in a freezer for years, lacking noteworthy degradation of phytochemicals.

Phenols are common and essential constituents of herbal material. They range in complexity from highly complex chemical compound substances to simple aromatic rings like flavonoids, anthraquinone, and coumarins. Synthetic resin substances are water-soluble and possess multiple biological effects and inhibitor activity. Gallic acid has been found that a bioactive agent acid that has powerful antihyperlipidemic effectivity and anti-hyperglycaemic in rats which can be facilitated through elevated levels of adiponectin in coexistence with attenuating tumor necrosis factor level and activated receptors of peroxisome proliferator up-regulation in fat tissue (Abdel-Moneim *et al.*, 2018). Since phenolic compounds, including gallic acid can lower blood sugar levels, the stress of oxidative protein glycation inhibits the act of dipeptidyl peptidase and some other crucial enzymes elaborate in metabolic actions, stimulating numerous biological routes to improve pancreatic-cell functions, increasing insulin secretion, and improving insulin sensitivity, they can be useful in diabetes management. Gallic acid may stimulate positive keratinocyte and fibroblast cell mobility and stimulate wound healing factors such as kinases of c-Jun N-terminal and focal adhesion, thereby continuing to support gallic acid's advantageous interference in wound repair caused by diabetes (Xu, Y *et al.*, 2021).

Flavonoids are powerful antioxidants with anti-inflammatory properties that have been accustomed to preventing diseases like polygenic disorders, cancer, and neurodegenerative diseases. Quercetin's working operations are pleiotropic, which involves inhibiting intestinal aldohexose absorption, secretory, insulin-sensitizing activities, and improved aldohexose utilization in peripheral tissues. Quercetin is well known for having antidiabetic movement. Recently testified quercetin can defend modifications in diabetic person during the stress of oxidative. Quercetin meaningfully endangered lipid-peroxidation as well as offers an antioxidant action in diabetes (Rizvi and Mishra, 2009). The antihyperglycemic impact of rutin embraces a decrease in carbohydrates preoccupation from the small-viscus, inhibition of tissue-gluconeogenesis, an increase in tissue aldohexose uptake, stimulation of internal exudation from cells of beta, and the Langerhans islets, which protects against degeneration. The research discovered that rutin, a flavonoid via antioxidant abilities, causes pancreatic islet regrowth and likely

increases insulin release in streptozocin-induced diabetic rats, thereby exerting its advantageous antidiabetic effects (Vessal *et al.*, 2003).

The impact of dietary tannins on people's well-being is a major issue, yet it additionally offers health-preventive advantages, as a few tannins are antioxidants. Phenolic compounds also alter enzymatic as well as transcriptional activities. Still, the capacity of nuclear receptors to regulate a wide variety of genes suggests that they could be used to extravagance diseases like diabetic complications as well as dyslipidemia. Receptors of nuclear are involved in the regulation of homeostasis of lipids. They determine a network of organized new metabolic process sensors that combine, among other things, the metabolism of lipids, inflammatory processes, the metabolism of drugs, bile acid synthesis, as well as the balance of glucose (Kumari and Jain, 2012).

Thin layer chromatographic of a particular leaf revealed various retention factor values in the range of 0.01 to 0.97. Here retention factor values in standard compounds 0.90, 0.50, 0.92, 0.44 are identified as gallic acid, rutin, quercetin, and tannic acid, respectively (Fig. 2). Retention factor values of leaves of in habitat *C. lanatus*, *T. foenum-graecum*, and *M. koenigii* are 0.90, 0.90, 0.90, identified as gallic acid in the Vadodara city plant sample, respectively. Where 0.90, 0.90, 0.91 which are identified as gallic acid in the Anand city plant sample, respectively (Fig. 2). Retention factor values of leaves of in habitat *C. lanatus*, *T. foenum-graecum*, and *M. koenigii* are 0.50, 0.50, 0.49, identified as rutin in the Vadodara city plant sample, respectively. Where 0.50, 0.50, 0.50 which are identified as rutin in the Anand city plant sample, respectively (Fig. 2). Retention factor values of leaves of in habitat *C. lanatus*, *T. foenum-graecum*, and *M. koenigii* are 0.91, 0.92, 0.92 which are identified as quercetin in the Vadodara city plant sample, respectively. Where 0.92, 0.92, 0.92 which are identified as quercetin in the Anand city plant sample, respectively (Fig. 2). Retention factor values of leaves in habitat *C. lanatus*, *T. foenum-graecum*, and *M. koenigii* are 0.44, 0.44, 0.43, identified as tannic acid in the Vadodara city plant sample, respectively. Where 0.44, 0.44, 0.44 which are identified as tannic acid in the Anand city plant sample, respectively (Fig. 2).

A high retention factor value clearly shows that the compound has traveled far up the plate and is, therefore, less polar, whereas a low retention factor value clearly shows that the compound has not traveled far and is, therefore, more polar (Bele and Khale, 2011). According to that, quercetin and gallic acid are less polar than tannic acid and rutin. Some spots are large and drifted like in quercetin in a sample of Vadodara of *C. lanatus* plant leaves because of handling errors like the plate not being perpendicular to the solvent when it was inserted into the chamber. All four compounds have a different therapeutic application, which is useful in treating different diseases. From the fruit rind of *P. granatum*, 0.90 retention factor values were identified as gallic acid (Al-Mosawe and Al-Saadi, 2012). From leaves of *E. neriifolia*, 0.92 and 0.49 retention factor values were identified as quercetin and rutin, respectively (Sharma and Janmeda, 2013). From *B. pinnatum*, 0.44 retention factor values were identified as tannic acid (Sharma *et al.*, 2013). As a result, the investigation we conducted was supported by previous investigations.



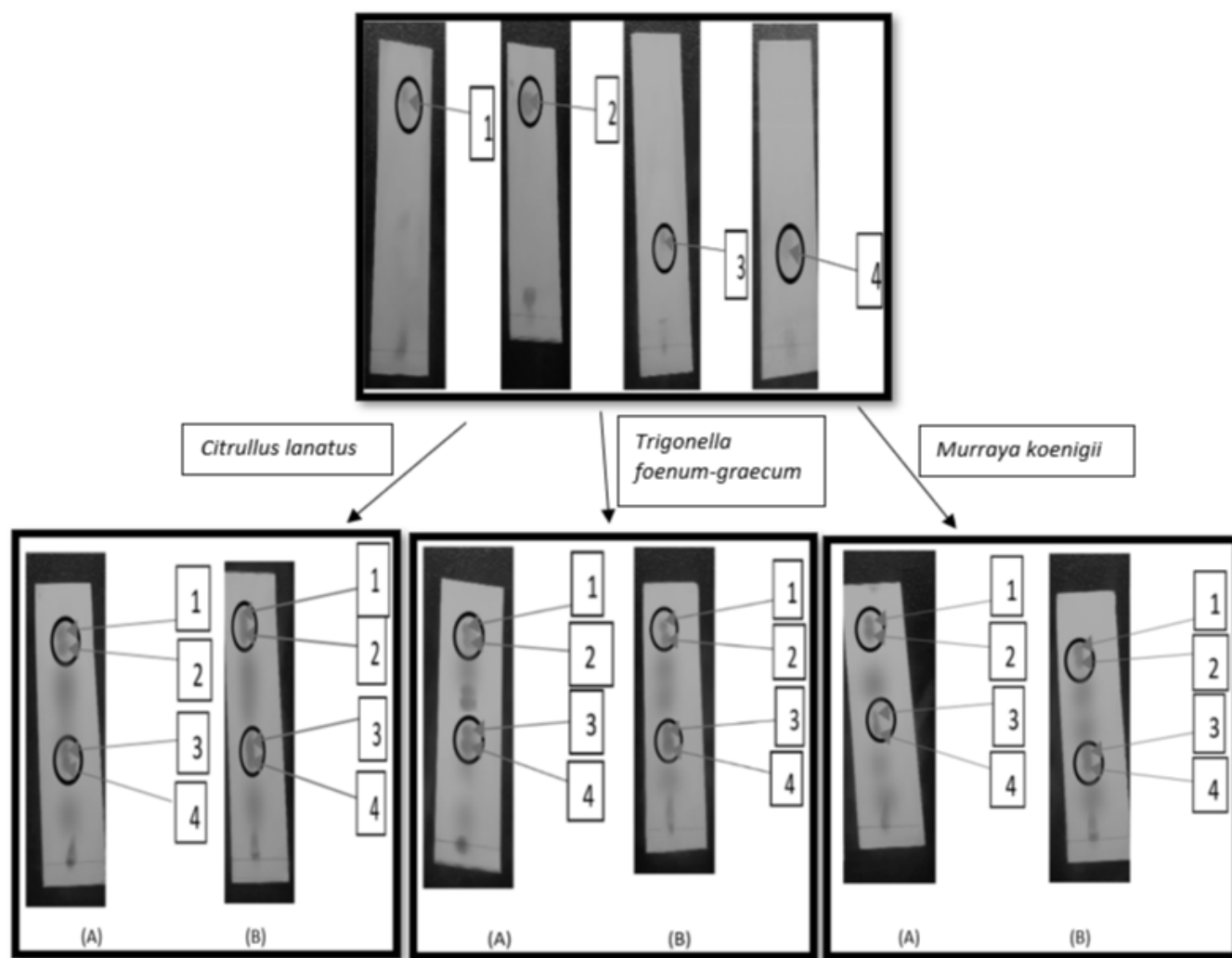


Fig 2: Thin layer chromatography profiling of compounds observed in plants by ultraviolet 254 nm. Where 1=Quercetin ; 2=Gallic acid; 3=Rutin; 4=Tannic acid and A=Vadodara ,B=Anand

### In-vitro antioxidant activity

#### Free radical scavenging activity using Diphenyl picryl hydrazyl (DPPH) free Radical

The diphenyl picryl hydrazyl free radical stability method is a simple, quick, and delicate technique for assessing the antioxidant activity of particular compounds and plant extracts. A steady 2, 2- diphenyl-1-picrylhydrazyl radical is employed in a simple method to assess the antioxidant function of plants. An odd electron inside the diphenyl picryl hydrazyl superoxide radicals generates a significant uptake maximum at 517nm, producing a color violet. The color changes from purple to yellow as the absorptivity of the Diphenyl picryl hydrazyl radical at 520 nm decreases. The subsequent reduction process is stoichiometric in terms of gaining electrons. A lower  $IC_{50}$  value shows greater antioxidant capacity. The greater the scavenging of diphenyl picryl hydrazyl radicals action, the lesser the  $IC_{50}$  values, indicating a strong antioxidant action. A lower  $IC_{50}$  value indicates improved diphenyl picryl hydrazyl radical scavenging ability (Ereifej *et al.*, 2016). Diabetes mellitus contributes to oxidative stress. Nitrogen and reactive oxygen species have

been delivered through a few endogenous and exogenous cycles, as well as cell reinforcement safeguards eliminate their detrimental effects. The imbalance between reactive oxygen besides nitrogen species creation and these cell reinforcement guards causes oxidative pressure. Individually, the concepts of reactive oxygen species and reactive nitrogen species relate to responding to extremist and non-revolutionary oxygen and nitrogen subsidiaries. Most vigorous cells recognize reactive oxygen and nitrogen species, which are vital in maturation only in age-related illnesses. It is becoming clear that oxidative play a vital role in advancing diabetes and the ensuing complications. Free radicals are usually generated by both exogenous and endogenous substances of the cell and their surroundings via the non-enzymatic oxidation process of organic compounds and responses begun by ionizing radiant energy. This communication may also occur in the mitochondrion via phosphorylation. Impairment of a body's defense system causes it to be unable to neutralize reactive oxygen species generation, likely to result in an impasse among reactive oxygen species as well as cell membrane protections, that also causes oxidative stress and therefore is detrimental to one's health (Asmat *et al.*, 2016).

In plants of antioxidant activity in Half-maximal inhibitory concentration of *M. koenigii*, *C. lanatus*, and *T. foenum-graecum* of Vadodara city  $80.28 \pm 2.12$ ,  $58.4 \pm 1.09$  and  $106.15 \pm 1.7$   $\mu\text{g/mL}$  higher than Anand city  $41 \pm 1.3$ ,  $28.72 \pm 0.05$ , and  $60.01 \pm 0.9$   $\mu\text{g/mL}$ , respectively. The standard ascorbic acid of antioxidant activity in Half-maximal inhibitory concentration is  $26.01 \pm 1.09$   $\mu\text{g/mL}$  (Fig 3). Compared to all six plants, the IC<sub>50</sub> value of *C. lanatus* of Anand city showed a  $28.72 \pm 0.05$   $\mu\text{g/mL}$  higher antioxidant action *present* (Fig 3). Our investigation has been supported by earlier investigations in leaves of *C. lanatus* and *M. koenigii* from both cities. It was reported that the antioxidant action of *C. lanatus* leaves was found at  $37.12$   $\mu\text{g/mL}$  (Aruna *et al.*, 2014).  $64.2 \pm 0.78$   $\mu\text{g/mL}$  (Devatkal *et al.*, 2012), as well as  $49.86 \pm 1.16$   $\mu\text{g/mL}$  (Vijayvargia and Vijayvergia, 2016) amount of antioxidant action was found in *M. koenigii*. Yet, there have been fewer studies conducted on *T. foenum-graecum* leaves beforehand. Also, it was reported that the antioxidant activity of *T. foenum-graecum* seed was found  $13.72 \pm 0.88$   $\mu\text{g/mL}$  (Saini *et al.*, 2016).

### In-vitro antidiabetic activity

#### Inhibition of alpha-amylase enzyme assay

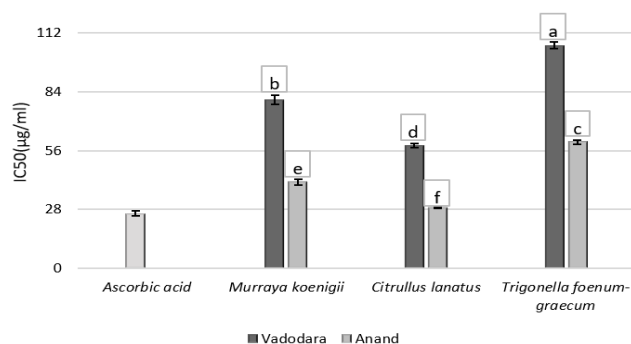
Pancreatic alpha-amylase is a member of the 1, 4-gluconohydrolase class and is an important objective enzyme in conventional diabetes management. It catalyzes the first stage inside the hydrolysis of starch to maltose and maltotriose, which are then broken down into glucose besides -glucosidases and absorbed into the bloodstream. Naturally occurring -amylase blockers derived from medicinal uses significant plants are very beneficial in controlling hyperglycemia, a serious issue in type 2 diabetes patients. Amylase has situated as an enzyme that disruptions bond through large-linked connections amid polysaccharides like glycogen as well as starch to produce glucose as well as maltose. Alpha-amylase blockers connect to polysaccharide alpha-bonds and help stop polysaccharide breakdown into mono and disaccharide. Drugs that hinder biological compound hydrolytic enzymes have been found to reduce hyperglycemia as well as enhance poor glucose metabolic activity in non-insulin-dependent people with diabetes without increasing the secretion of insulin. Alpha-amylase is indeed an enzyme involved in the first step of starch

hydrolysis, which serves as a major glucose source inside the diet. Alpha-amylase inhibitors are representatives that impede bioactivity, having caused carbohydrate absorption to be delayed as well as the as a whole simple sugars absorption of nutrients duration to be prolonged, resulting in a decrease in the rate of glucose uptake as well as, as a result, a decrease in the postprandial plasma glucose increase (Tundis *et al.*, 2010).

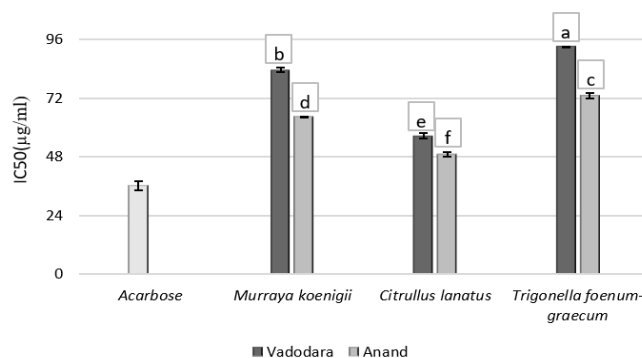
In plants of alpha-amylase enzyme assay in a half-maximal inhibitory concentration of *M. koenigii*, *C. lanatus*, and *T. foenum-graecum* of Vadodara city  $83.55 \pm 1.00$ ,  $56.62 \pm 1.02$  and  $92.91 \pm 0.17$   $\mu\text{g/mL}$  higher than Anand city  $64.35 \pm 0.08$ ,  $49.11 \pm 0.9$  and  $73.06 \pm 1.1$   $\mu\text{g/mL}$ , respectively. The standard acarbose of alpha-amylase enzyme assay in half-maximal inhibitory concentration is  $36.21 \pm 2.01$   $\mu\text{g/mL}$  (Fig 4). Compared to all six plants, *C. lanatus* of Anand city showed a  $49.11 \pm 0.9$   $\mu\text{g/mL}$  higher antidiabetic action *present* (Fig 4). The investigation we conducted has been supported through earlier investigations in *M. koenigii* as well as *C. lanatus* of both cities. It was reported that the antidiabetic action of *M. koenigii* was found at  $63.28$   $\mu\text{g/mL}$  (Narkhede, 2012).  $31.32$   $\mu\text{g/mL}$  amount of antidiabetic action was found in *T. foenum-graecum* leaves (Narkhede, 2012). The results we obtained verify previous investigations in the leaves *T. foenum-graecum* from Anand, yet not from Vadodara. Also, it was reported that the antidiabetic activity of *C. lanatus* peel was found at  $72.15$   $\mu\text{g/mL}$  (Sani and Nair, 2017), and in leaves, it was found at  $58.558$   $\mu\text{g/mL}$  (Aruna *et al.*, 2014). These differences in outcomes may be attributed to all of the parameters, such as the number of nutrients in the soil, geological region, and environment as well as other factors such as latitude, temperature, longitude, rainfall, and quality of soil (Yadav, 2018).

#### Inhibition of alpha-glucosidase enzyme assay

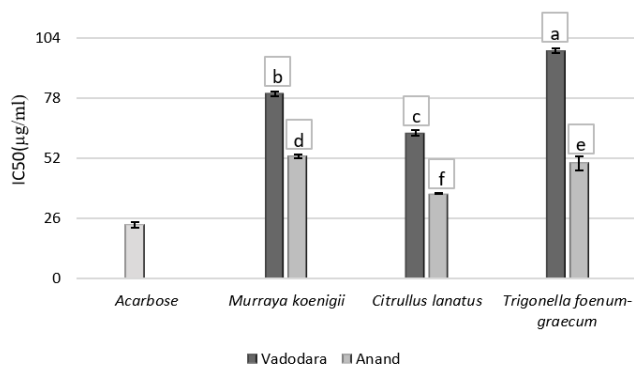
Alpha-Glucosidase antagonists are employed to improve blood sugar control of high blood sugar through type two diabetes, especially postprandial hyperglycemia. They can be taken as combination therapy through combination with insulin-dependent healthy eating and exercise or even in combination with other antidiabetic medications. Alpha-glucosidase inhibitors could also be beneficial to type 2 diabetes patients. Controlling postprandial blood sugar levels seems essential in the disease's initial treatment. Among the treatment



**Fig 3:** Antioxidant activity of *Murraya koenigii*, *Citrullus lanatus* and *Trigonella foenum-graecum* respectively. All the values are expressed as Mean  $\pm$  SD (Standard Deviation) (n=3) and are significantly different at  $p < 0.05$ . <sup>abcdef</sup> Values with different letter above the bas for a given extract are significantly different from each other (Tukey's HSD and Duncan multiple range post-hoc tests, IBM SPSS for Windows).



**Fig 4:** Alpha-amylase enzyme assay of *Murraya koenigii*, *Citrullus lanatus* and *Trigonella foenum-graecum* respectively. All the values are expressed as Mean  $\pm$  SD (Standard Deviation) (n=3) and are significantly different at  $p < 0.05$ . <sup>abcdef</sup> Values with different letter above the bas for a given extract are significantly different from each other (Tukey's HSD and Duncan multiple range post-hoc tests, IBM SPSS for Windows).



**Fig 5:** Alpha-glucosidase enzyme assay of *Murraya koenigii*, *Citrullus lanatus* and *Trigonella foenum-graecum* respectively. All the values are expressed as Mean  $\pm$  SD (Standard Deviation) (n=3) and are significantly different at  $p < 0.05$ . <sup>abcdef</sup> Values with different letter above the bar for a given extract are significantly different from each other (Tukey's HSD and Duncan multiple range post-hoc tests, IBM SPSS for Windows).

interventions for lowering glucose production is indeed the potent inhibitor of enzyme systems through glycogen synthesis. Alpha-glucosidase is an enzyme that aids the digestion process. It encourages the rise of blood glucose levels within a week of meals by rapid hydrolysis of 1, 4-glucosidic bonds inside of carbohydrate consumption. Alpha-glucosidase inhibitors hinder alpha-glucosidase action, postponing small intestine carbohydrate digestion and trying to slow the dramatic increase in blood glucose levels which people with diabetes commonly experience after lunch and dinner. As just a result, alpha-glucosidase blockers like acarbose are medically used as antihyperglycemic substances. Even so, they frequently cause serious gastrointestinal problems. As just a result, the research intended for novel alpha-glucosidase inhibitors from plant sources has emerged as an appealing method for treating the risk of developing diabetes (Gong *et al.*, 2020).

In plants of alpha-glucosidase enzyme assay in Half-maximal inhibitory concentration of *M. koenigii*, *C. lanatus*, and *T. foenum-graecum* of Vadodara city  $79.95 \pm 0.99$ ,  $62.99 \pm 1.21$  and  $98.49 \pm 1.2$   $\mu\text{g/mL}$  higher than Anand city  $52.75 \pm 0.8$ ,  $36.81 \pm 0.33$ , and  $49.91 \pm 3.07$   $\mu\text{g/mL}$  respectively. The standard acarbose of alpha-glucosidase enzyme assay in half-maximal inhibitory concentration is  $23.33 \pm 1.11$   $\mu\text{g/mL}$ . Compared to all six plants *C. lanatus* of Anand city showed a  $36.81 \pm 0.33$   $\mu\text{g/mL}$  higher antidiabetic action *present* (Fig 5). In the leaves of all three plants, our findings outperformed previous research. It was reported that the antidiabetic activity of *M. koenigii* was found 174.74  $\mu\text{g/mL}$  (Gul *et al.*, 2012). 627.270  $\mu\text{g/mL}$  amount of antidiabetic action was found in *C. lanatus* leaves (Aruna *et al.*, 2014). This demonstrates that all of our plant's leaves have antidiabetic properties.

## CONCLUSION

The present study indicates that *C. lanatus*, *T. foenum-graecum*, and *M. koenigii* plants, specifically leaves have countless therapeutic applications. The present study confirms that all three plants have common class of compounds identified as rutin, gallic acid, quercetin, and tannic acid, which have proven to have a great deal of medicinal activity which is very rare and

unique in any medicinal plant. Also, all three plant leaves show antioxidant and antidiabetic activities but *C. lanatus* from Anand city was found to be the best among plants. The presence of metabolites and their antioxidant and antidiabetic properties significantly promote all three plant leaves for their beneficial use in medicine and pharmaceuticals as potent drugs used to cure a variety of ailments. Therefore, the present research has presented supportive information on profitable concerns in research organizations and pharmaceutical companies for developing new drugs for dealing with several complications.

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## AUTHOR'S CONTRIBUTION

Richa Dodia doing Ph.D. in botany from Charutar vidya Mandal University, Anand, Gujarat. In the current study, she has contributed to the writing and compilation of data in this manuscript.

Susmita Sahoo has received her Ph.D. in botany from IMMT (CSIR) Bhubaneswar & Utkal University. She is currently a Senior Assistant Professor at Charutar Vidhya Mandal University, Vallabh Vidyanagar, Anand, Gujarat. In the current study, she was involved in the guidance of literature collection and manuscript preparation.

## CONFLICT OF INTEREST

We have no conflicts of interest to disclose

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