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Enzyme-Based *In Vitro* Assessment of the Hypoglycaemic Potential of *Lamiaceae* Leafy Spices

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Abstract

Background: The increasing global prevalence of Diabetes Mellitus type 2 (DM II) has prompted the search for safer and more effective alternatives to synthetic antidiabetic drugs. The management of postprandial hyperglycaemia through the inhibition of carbohydrate-digesting enzymes, particularly α-amylase and α- glucosidase, has emerged as an effective therapeutic strategy. Plants belonging to the Lamiaceae family have attracted considerable attention as potential sources of bioactive compounds for the treatment of metabolic disorders, including diabetes. However, comprehensive *in vitro* studies validating their efficacy as anti-hyperglycemic agents remain limited. **Objectives:** The present investigation was undertaken to evaluate and compare the hypoglycemic potential of selected Lamiaceae leafy spices through *in vitro* enzyme inhibition assays. **Methods:** Methanolic and aqueous extracts were prepared and evaluated for their inhibitory effects against carbohydrate digesting enzymes. The inhibitory concentration (IC_{50}) values were determined from plots of percent inhibition versus the logarithmic inhibitor concentration and were calculated by non- linear regression analysis using the mean inhibitory values. Acarbose was used as the standard reference inhibitor for both the assays. **Results:** The findings suggest that all the tested spices exhibited significant *in vitro* hypoglycaemic potential, highlighting their possible application as promising alternative therapeutic agents for the management of DMII. **Conclusion:** The selected leafy spices belonging to the Lamiaceae family have long been utilized in traditional culinary and medicinal systems and are recognized for their richness in phenolic compounds. The present study revealed their positive effects on glycaemic control. The findings further underscore the growing interest in the utilization of spice-derived phytochemicals in functional foods, nutraceuticals and contemporary therapeutic formulations. Nevertheless, further research and clinical validation are required to confirm their efficacy, long-term safety and translational potential in the management of Diabetes Mellitus type 2.

Keywords
α-amylase, α- glucosidase, Hypoglycaemic potential, *In vitro* assays, Lamiaceae, Leafy spices, Phenolic compounds.

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1. INTRODUCTION

Approximately 80% of the global diabetic population resides in developing countries, with India being recognized among the top ten nations with the highest prevalence of diabetes mellitus [1]. Type 2 Diabetes Mellitus (DM II) is a condition characterized by an imbalance between insulin secretion and blood sugar absorption. It is a chronic metabolic and endocrine disorder characterized by hyperglycaemia and glucose intolerance [2]. Postprandial hyperglycemia is one of the important factors in the progression of diabetes and its associated complications like nephropathy, retinopathy and neuropathy, delayed wound healing, heart attack,

peripheral vascular disturbances and ketoacidosis [3]. The consumption of synthetically derived medicines such as metformin and other sulfonylureas leads to damage to body organs. Consequently, there has been growing scientific interest to look for bio- based active principles with anti-diabetic properties.

Medicinal and aromatic plants have long been utilized for the treatment of various diseases and constitute an important component of traditional healthcare systems [4]. Indigenous communities in the Brazilian Amazon have traditionally utilized plants belonging to the Lamiaceae to manage diabetes mellitus [5]. Previous studies have reported that around 115 medicinal plants exhibit antidiabetic effects, mainly from the Fabaceae, Lamiaceae and Liliaceae families [6]**.** In particular, culinary herbs and spices of the Lamiaceae family have emerged as promising sources of therapeutic agents for nutritional and health benefits and for the management of metabolic disorders, including diabetes [7-10].

Bioactive compounds like alkaloids, triterpenoids, flavonoids and phenolics are known to play important role in combating DM II [11]. Furthermore, kaempferol, fenugreekine and ar-turmerone are phytochemicals present in these spices, have been known as potent anti-diabetic compounds [12]. They exert beneficial effects through modulation of various biological pathways, including oxidative stress, inflammatory cascades and lipid metabolism. The present work highlights the potential of culinary spices in the development of functional foods, preventive nutritional strategies and sustainable food systems. Such studies may also contribute to the discovery of novel plant-derived therapeutic agents that support sustainable and holistic healthcare practices.

1.1 Potential mechanisms of anti-hyperglycaemic action and glucose homeostasis

The anti-diabetic mechanisms reported under this category include inhibition of carbohydrate hydrolysing enzymes, increase in glucose transport across the membrane, stimulation of insulin secretion and decrease in pancreatic β-cell damage, activation of insulin receptors, enhancement of pancreatic beta-cell regeneration and regulation of hepatic glucose production [13-18]. Flavonoids have also been shown to enhance insulin release from pancreatic islets and suppress glucose levels [19].

Nowadays, drug and dietetic therapies are the two major approaches used for prevention and control of DM II [20]. Among different therapeutic strategies, the inhibition of carbohydrate-digesting enzymes—particularly α-amylase and α-glucosidase by dietary components or therapeutic agents has been recognized as an effective approach to slow down glucose absorption and to control postprandial hyperglycaemia [21].

1.2 Phytoconstituents and pharmacological profile of Lamiaceae leafy Spices

Various studies have reported that plants rich in flavonoids, phenols and tannins exhibit significant anti-diabetic activity. Phenolic compounds and tannins may contribute to this effect through multiple mechanisms, including insulin-mimetic activity, stimulation of insulin secretion, protection of pancreatic β-cells against oxidative damage, promotion of β-cell regeneration and proliferation and reduction of carbohydrate absorption via inhibition of α-amylase and α-glucosidase enzymes. Flavonoids are known to interact with various molecular targets involved in glucose homeostasis by enhancing β-cell growth, stimulating insulin release, preventing cellular apoptosis and regulating hepatic glucose metabolism, thereby contributing to the control of hyperglycemia [22].

The phytochemistry and pharmacological properties, including the anti-diabetic activities of different members of this family have been explored and the active principle derived from these species have demonstrated potential for use in the treatment of DMII. The therapeutic applications of the *Lamiaceae* are mainly related to its essential oils, which have been reported to contribute to a wide range of biological activities [23, 24]. For instance, the essential oil extracted from *Mentha pulegium* has demonstrated significant α-amylase and α-glucosidase inhibitory activities [25]. Species of *Salvia* are also rich in essential oils containing major bioactive constituents such as α-pinene, β-pinene, limonene, linalool, caryophyllene, germacrene, myrcene, α-thujone, and humulene. These phytochemicals have also been associated with wide range of pharmacological activities, including anticancer, antioxidant, anti-diabetic, antibacterial, anti-inflammatory, antithrombotic, anti-rheumatic and antiviral properties [26, 27]. Apigenin isolated from *Salvia coccinea* has been reported to exhibit hypoglycemic potential through multiple mechanisms, including reduction of α-amylase activity, cholesterol levels, insulin resistance and DRP1 (Dynamin Related Protein 1, a GTPase protein) expression. In addition, it enhances the expression of GLUT4 (Glucose Transporter Type 4), GSK-3β (Glycogen Synthase Kinase-3β) and signaling pathways such as AMPK (AMP-activated protein kinase) /PI3K (Phosphoinositide 3-kinase) /Nrf2 (Nuclear factor erythroid2-related factor 2) and PI3K-Akt (Phosphoinositide 3-kinase-Protein kinase B),

which are mainly involved in glucose metabolism [28]. According to a review article, eugenol present in *Ocimum* species may help regulate glucose metabolism, reduce oxidative stress and improve insulin sensitivity [29]. Similarly, *Hyssopus officinalis* has been reported to contain a wide range of biologically active compounds, including flavonoids such as apigenin and luteolin, essential oils and several polyphenolic compounds including chlorogenic, ferulic, caffeic, p-hydroxybenzoic, catechuic, vanillic, p-coumaric and rosmarinic acids [30, 31]. Similarly 27 bioactive compounds have been identified in the methanolic extract of *Thymus vulgaris* [32]. Thymol, a monoterpene derivative of cymene and an isomer of carvacrol, is recognized for its antioxidant activity [33]. In addition to thymol, linalool, carvacrol, and p-cymene have also been identified as major components in the leaf extract and are considered responsible for anti-diabetic and anticancer properties [34]. Bioactive compounds such as curcumin, resveratrol and berberine, have also shown protective and regenerative effects on β-cells [35]. Rosmarinic acid has demonstrated inhibitory effects towards α- glycosidase enzyme associated with DMII [36]. Moreover, naturally occurring carbazole alkaloid, mahanimbine, isolated from Lamiaceae members has been identified as an important anti-diabetic compound and has been studied for its hypolypidemic activity in streptozotocin- induced diabetic rats [37]. Several bioactive polyphenols and flavonoids, including origanol, ursolic acid, oleanolic acid, β-sitosterol and triacontanol were isolated from the methanolic extract of the leaves of *Origanum vulgare* and proved to be safe and effective against diabetes [38].

1.3 *In vitro* and *in vivo* biological investigations of Lamiaceae leafy spices

Several studies have investigated the *in vitro* antioxidant, α-amylase and α-glucosidase inhibitory potential of Lamiaceae leafy spices including *Cinnamomum tamala* [39], *Rosmarinus officinalis* [40] and *Ajuga integrifolia* [22]. Likewise, in *H. officinalis*, α-glucosidase inhibitory activity was observed in the aqueous extract of dried leaves [41]. *R. officinalis* leaf aqueous extracts were found to contain substantial amounts of phenolics, flavonoids and tannins, which exhibited strong *in vitro* DPPH free radical scavenging activity [42]. Comparable antioxidant and antidiabetic effects have also been reported for methanolic leaf extracts of *O. vulgare*. *In vitro* antioxidant, neuroprotective and anti-diabetic activities of essential oils obtained from eight members of the Lamiaceae, including lavender, lemon, myrtle, oregano, peppermint, sage and rosemary leaves, have been investigated. The study revealed that antioxidant activity was positively associated with phenolic content, while α-amylase inhibitory activity showed a strong correlation with antioxidant potential [8].

Clinical evidence supporting the therapeutic efficacy of leafy spices in humans remains limited. However, several *in vivo* investigations have demonstrated promising anti-diabetic potential of these plants. A 90% ethanolic extract of *C. tamala* leaves showed significant hypoglycemic activity in both normal and diabetic male albino rats. The observed effect was attributed to enhanced peripheral glucose utilization and increased muscle glycogen storage, possibly mediated through stimulation of insulin secretion from pancreatic β-cells [43]. Similarly, ethanolic leaf extracts of *Origanum majorana* demonstrated significant antidiabetic and antihyperlipidemic activities in streptozotocin-induced diabetic rats [44]. Aqueous extracts of *M. koenigii*, *Mentha spicata* and *T. vulgaris* leaves demonstrated neuroprotective potential in streptomycin induced diabetic rats, thereby providing a biochemical basis for future clinical investigations.

Dietary supplementation with leaf powder of *R. officinalis* significantly reduced blood glucose levels in streptozotocin-induced diabetic rats [42]. Ethanolic extracts of the plant also showed notable α-amylase inhibitory and anti-hyperglycaemic activities. Furthermore, *in vivo* studies investigating the anti-diabetic, hypolipidemic and anti-oxidative effects of *Salvia officinalis* leaves in streptozotocin-induced type 2 diabetic Wistar albino male rats revealed significant protective effects against diabetes-associated oxidative stress. Fermented camel milk enriched with *S. officinalis* extract was also found to confer protection against diabetes and oxidative damage. Additionally, *S. officinalis* enhanced GLUT4 expression, thereby contributing to improved glucose homeostasis [45].

Enhanced anti-diabetic efficacy has also been reported for selenium nanoparticles synthesized using a combination of *Cinnamomum verum, O. majorana* and *O. vulgare*. Among these, *O. vulgare* has been shown to inhibit glycosylation and α-glucosidase activity, enhance glucose uptake and reduce oxidative stress [46].

In vitro and *in vivo* studies have revealed that several members of Lamiaceae, including the leafy spices possess notable hypoglycaemic potential. The observed effects may be attributed to the presence of polyphenols and other secondary metabolites that inhibit enzymatic carbohydrate digestion, thereby potentially reducing postprandial glucose elevation.

2. MATERIALS AND METHODS

In Indian cuisine fruits and seed spices are generally consumed in dried form, whereas leafy spices are utilized in both fresh and dried forms. Therefore, the present study was designed to evaluate and compare the antihyperglycemic potential of Lamiaceae leafy spices in their fresh as well as dried forms. The *in vitro* evaluation of anti-hyperglycaemic efficacy of *Ocimum basilicum* L. (Basil) *Hyssopus officinalis* L. (Hyssop), *Marjorana hortensis* Moench. (Marjoram), *Mentha piperita* L.(Mint), *Origanum vulgare* L.(Oregano), *Rosmarinus officinalis* L.(Rosemary), *Salvia officinalis* L.(Sage), *Satureja hortensis* L. (Savory) and *Thymus vulgaris* L. (Thyme) was carried out.

The spice samples were procured from Alleppey outlet of Spices Board India. The collected samples were assigned numbers ranging from BNU/01/2023/01 to BNU/01/2026/09 and submitted in Department of Botany, B N P G College, Faculty of Science, B N University, Udaipur (Rajasthan) for further reference.

Methanolic (EE) and aqueous extracts (AE) of the selected spices were prepared and evaluated for their inhibitory effects against α-amylase and α-glucosidase using standard experimental protocols. The control samples were prepared without any spice extract and absorbance was recorded at 540 nm. The percentage inhibition was calculated according to the method prescribed by Jung and co-workers [47]. The inhibitory concentration (IC_{50}) values were determined from plots of percent inhibition versus logarithmic inhibitor concentration and were calculated by non- linear regression analysis using the mean inhibitory values. Acarbose was used as the standard reference inhibitor for both, α-amylase and α-glucosidase assays. All tests were carried out in triplicate. The experimental data were expressed as mean ± standard error of the mean (SEM) obtained from three replicates. A p-value < 0.001 was considered statistically highly significant.

3. RESULTS AND DISCUSSION

Hypolipidemic efficiency of the AE and EE extracts of the selected Lamiaceae leafy spices:

3.1 α-glucosidase and α -amylase inhibitory activity of fresh leafy spices. (Table 1.)

Highest α-glucosidase inhibition in aqueous extract was observed by *O. basilicum* (48.73%) followed by *T. vulgaris* (44.64%), *S. officinalis* (40.84%), while *R. officinalis* (35.45%), *S. officinalis* (29.4%) *and T. vulgaris* (27.64%) in methanolic extract. α -amylase inhibitory activity was observed maximum by *O. basilicum*(46.5%) followed by *T. vulgaris* (41.89%) and *S. officinalis* (37.34%) in aqueous phase while *R. officinalis* (31.8%) followed by *O. basilicum* (22.5%) and *S. hortensis* (21.65%) in methanolic extract. Comparison of percent α-glucosidase inhibition between aqueous extract and methanolic extract of fresh leafy spices, data reveals promising inhibition in aqueous phase as compared to methanolic except in *H. officinalis.* In aqueous forms as compared to methanolic extract more than fifty percent inhibition efficacy was observed in *M. piperita* (56.61%), *O. basilicum* (51.82%) and *O. vulgare* (52.72%). Comparison of percent α-amylase inhibition between aqueous extract and methanolic extract of fresh leafy spices, data reveals the similar pattern as of α-glucosidase inhibition.

Table 1: α-glucosidase and α -amylase inhibitory activity of leaves (fresh form) used as spices

Spice	AGI (A) (%)	AGI (M) (%)	AAI (A) (%)	AAI (M) (%)
Hyssopus officinalis	4.84 ± 0.86**	5.66 ± 0.12**	3.8 ± 1.33**	4.0 ± 0.66***
Marjorana hortensis	24.53 ± 0.50***	17.77 ± 1.15	21.45 ± 2.00*	13.8 ± 0.12**
Mentha piperita	3.78 ± 1.00	1.64 ± 0.10***	2.1 ± 0.38	1.67 ± 0.86
Ocimum basilicum	48.73 ± 0.86	23.48 ± 0.12	46.5 ± 0.66***	22.5 ± 1.00
Origanum vulgare	4.23 ± 0.50***	2.0 ± 0.12	3.8 ± 1.33	1.8 ± 0.38
Rosmarinus officinalis	36.78 ± 0.66**	35.45 ± 0.50	32.0 ± 0.12**	31.80 ± 1.75
Salvia officinalis	40.84 ± 1.00	29.4 ± 2.15***	37.34 ± 0.66	21.0 ± 0.12
Satureja hortensis	27.0 ± 0.12	22.24 ± 0.86	23.45 ± 1.75	21.65 ± 2.15*
Thymus vulgaris	44.64 ± 1.75**	27.64 ± 0.66	41.89 ± 1.86	20.84 ± 0.90*

*AGI(A): α-glucosidase inhibition (Aqueous Extract); AGI(M): α-glucosidase inhibition (Methanolic Extract); AAI(A): α-amylase inhibition (Aqueous Extract); AAI(M): α- amylase inhibition (Methanolic Extract). The activity of each spice was tested using 100 ml aqueous or methanol extract, corresponding to 1 mg dry spice. Values are mean ± SEM and P *<0.05; **<0.01; ***<0.001*

3.2 α-glucosidase and α -amylase inhibitory activity of dried leafy spices. (Table 2.)

Maximum inhibition of α-glucosidase activity was observed in *T. vulgaris* (44.0%) followed by *S. officinalis* (40.4%), *O. basilicum* (36.87%) *and R. officinalis* (35.0%) in aqueous phase, the same was also observed in methanolic extract except for *O. basilicum* (8.09%). Highest α-amylase inhibition in studied dried leafy spice was observed in *T. vulgaris* (41.0%) followed by *S. officinalis* (39.89%), *O. basilicum* (32.9%) and *S. hortensis* (29.84%) in aqueous extract while in methanolic form the inhibitory progression included *T. vulgaris* (38.75%), *S. officinalis* (23.4% and, *R. officinalis* (19.0%). α-glucosidase and α -amylase inhibitory activity was nearly equal by *M. piperita* in both the extract. Inhibitory efficacy of *T. vulgaris* for both the enzymes in both extracts was nearly equal revealing its efficacy in post prandial regulation.

Table 2: α-glucosidase and α -amylase inhibitory activity of leaves (dried form) used as spices

Spice	AGI (A) (%)	AGI (M) (%)	AAI (A) (%)	AAI (M) (%)
Hyssopus officinalis	6.32 ± 1.33**	2.33 ± 0.10	4.32 ± 0.50	1.8 ± 1.15
Marjorana hortensis	18.34 ± 1.15	7.65 ± 0.33	14.56 ± 0.86	5.00 ± 1.00**
Mentha piperita	3.8 ± 0.66	3.1 ± 1.00	2.95 ± 0.12***	2.9 ± 0.38
Ocimum basilicum	36.87 ± 2.15***	8.09 ± 0.33	32.9 ± 0.66	10.0 ± 1.00
Origanum vulgare	4.21 ± 0.12	1.23 ± 0.50	3.6 ± 1.00	1.35 ± 0.38*
Rosmarinus officinalis	35.0± 0.66***	21.67 ± 0.12	31.5 ± 0.86	19.0 ± 0.76**
Salvia officinalis	40.4 ± 1.00	21.5 ± 1.75	39.89 ± 0.33***	23.4 ± 1.15
Satureja hortensis	32.48 ± 0.12*	17.8 ± 1.26**	29.84 ± 1.15	14.5 ± 0.50
Thymus vulgaris	44.0 ± 0.66*	41.8 ± 0.86	41 ± 1.33***	38.75 ± 0.12

*AGI(A): α-glucosidase inhibition (Aqueous Extract); AGI(M): α-glucosidase inhibition (Methanolic Extract); AAI(A): α-amylase inhibition (Aqueous Extract); AAI(M): α- amylase inhibition (Methanolic Extract). The activity of each spice was tested using 100 ml aqueous or methanol extract, corresponding to 1 mg dry spice. Values are mean ± SEM and P *<0.05; **<0.01; ***<0.001*

3.3 Comparison of α-glucosidase and α -amylase inhibition of fresh and dried leafy spices in aqueous extract

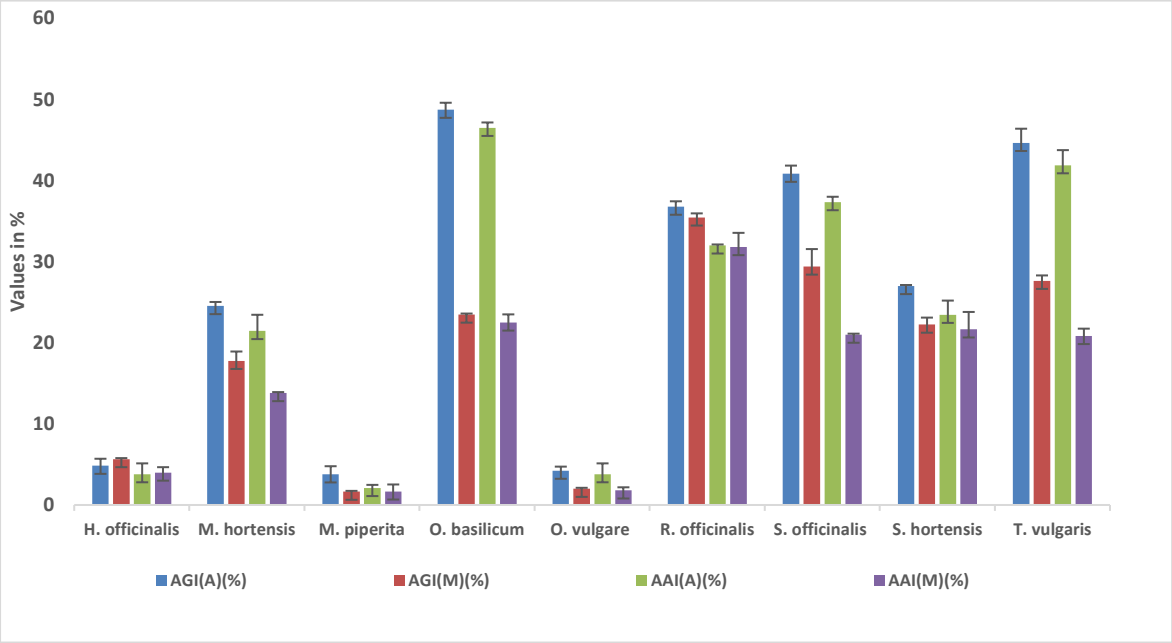


Figure 1: Comparative values of α-glucosidase inhibition and α-amylase inhibition activity of Lamiaceae leafy spices in fresh form in aqueous and methanolic phases.

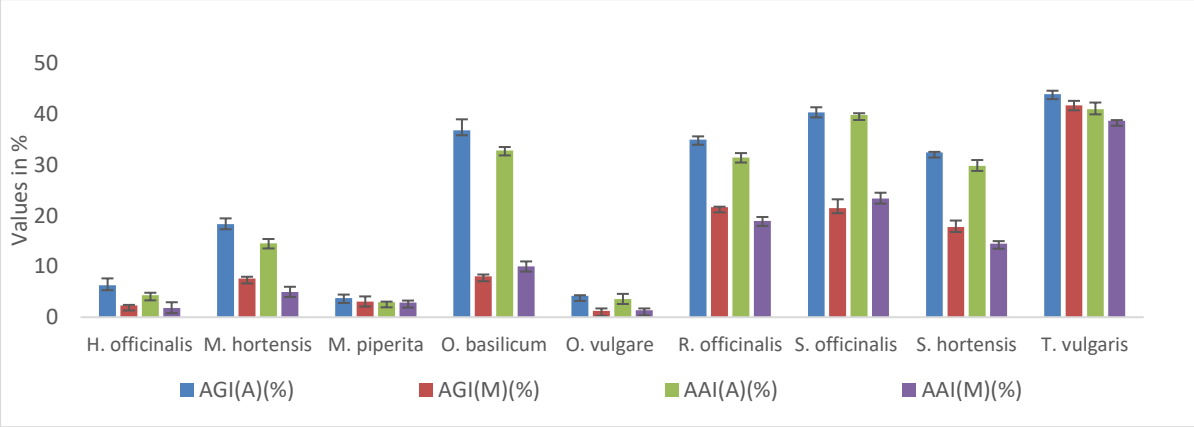


Figure 2: Comparative values of α-glucosidase inhibition and α-amylase inhibition activity of Lamiaceae leafy spices in dried form in aqueous and methanolic phases.

In aqueous extracts percent α-glucosidase inhibition was comparatively more in fresh leafy spices as compared to dried except in *H. officinalis, and S. hortensis* and nearly equal inhibition by *M. piperita, O. vulgare, S. officinalis* and *T. vulgaris.* The same lineage was also observed for percent α-amylase inhibition where comparatively high inhibition was shown by fresh forms except *H. officinalis, M. piperita, S. officinalis* and *S. hortensis.* Negligible inhibition by both fresh and dried forms and for both the enzymes was observed in *H. officinalis, M. piperita and O. vulgare* revealing their lack of efficacy in post prandial hyperglycaemia and in turn poor anti-diabetic efficiency. Richly inhibition for both the enzymes by *O. basilicum, T. vulgaris, S. officinalis, R. officinalis* and *S. hortensis* indicates their effective usage for post prandial hyperglycaemia regulation and promising hypoglycaemic spices. In particular, the aqueous extracts showed the highest inhibitory activity against both the enzymes. Similar results were observed previously in a study, where aqueous extracts of *R. officinalis* leaf were found to contain high amount of flavonoids, phenolic compounds and tannins and thus exhibited strong *in vitro* free radical scavenging activity [42].

The findings of the present study are consistent with previous reports demonstrating the hypoglycemic potential of leafy spices belonging to the Lamiaceae family through the inhibition of α-amylase and α-glucosidase enzymes. Similar enzyme inhibitory and antihyperglycemic activities have been documented in *A. integrifolia* [22], *C. tamala* [39], *H. officinalis* [41], *O. vulgare* [46], *R. officinalis* in both *in vitro* [40] and *in vivo* [42] studies and in *S. officinalis* [45], and in *S. coccinea* [28].

Species of Lamiaceae family produce wide range of secondary metabolites, including bioactive compounds present in essential oils. The essential oils have been reported to exhibit antioxidant potential [24]. Several studies employing *in vitro* and *in vivo* methods have revealed that the antioxidant activity of leafy spice is positively associated with their phenolic content, while α-amylase and α-glucosidase inhibitory activities are strongly correlated with their antioxidant potential [8, 48, 49]. Thus, dietary polyphenols act as antidiabetic agents [14].

4. CONCLUSION

Among the various therapeutic approaches for the management of Type 2 Diabetes Mellitus, inhibition of carbohydrate-digesting enzymes represents one of the most effective strategies for controlling postprandial hyperglycemia. In the present study, ethanolic and aqueous extracts of selected leafy spices were evaluated through *in vitro* α-amylase and α-glucosidase enzymatic assays. The findings suggest that among all the studied dried leafy spices, α-glucosidase inhibition was more prominent than α-amylase inhibition in the aqueous medium. In the aqueous phase, marked inhibition of both the enzymes was observed in *T. vulgaris, S. officinalis, O. basilicum and S. hortensis* while *O. vulgare, M. piperita* and *H. officinalis* exhibited comparatively lower inhibitory activity. According to research review they also possess notable antioxidant and anti-inflammatory properties, which may contribute to their anti-hyperglycemic effects through multiple mechanisms.

These findings suggest that all the tested Lamiaceae leafy spices exhibit significant *in vitro* antidiabetic potential, highlighting the promising potential of these spices as alternative therapeutic agents and encourage the development of safer bio-based drugs for diabetes management. Nevertheless, further experimental investigations and well-designed

clinical trials are required to confirm their efficacy, safety and mechanisms of action in the treatment of diabetes mellitus.

5. CHALLENGES AND FUTURE DIRECTIONS

To date, numerous spice extracts have been successfully investigated for the management of Type 2 Diabetes Mellitus. Leafy spices belonging to the Lamiaceae family possess considerable potential to emerge as effective alternative therapeutic agents owing to their accessibility, affordability and therapeutic efficacy. Although these spices have demonstrated beneficial effects in reducing postprandial glucose levels, the precise mode of action of their bioactive compounds still remain complex and insufficiently understood. This complexity arises from the presence of a wide variety of phytochemicals within each spice, which may exert synergistic interactions.

Future research should therefore focus on the isolation and characterization of active phyto-constituents, along with detailed investigation of the molecular mechanisms and signaling pathways underlying hypoglycemic activity. Evaluation of therapeutic potential of these bioactive compounds with respect to efficacy, safety, bioavailability and possible adverse effects is essential to ensure their safe and effective therapeutic application.

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