EVALUATION OF ANTIDIABETIC EFFECT OF ETHANOLIC EXTRACT OF MINT FRESH POWDER, CARBON AND ASH

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ABSTRACT

The present study investigated the phytochemical composition, antioxidant capacity, and antidiabetic potential of mint (*Mentha spicata*) leaves in three different forms: ethanolic extract of fresh powder, carbon dots, and ash. Preliminary phytochemical screening of the ethanolic extract revealed the presence of alkaloids, carbohydrates, phenols, flavonoids, sterols, diterpenoids, and quinones. Quantitative analysis demonstrated that phenols and flavonoids were present in higher concentrations compared to alkaloids and tannins, indicating their possible contribution to bioactivity. Antioxidant activity was evaluated using ABTS, DPPH, nitric oxide, and ferric ion scavenging assays, where significant radical scavenging activity was observed, particularly attributable to phenolic and flavonoid constituents. The antidiabetic potential of mint leaves was assessed through inhibition of key carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase. The ethanolic extract of fresh mint powder showed notable enzyme inhibition in a concentration-dependent manner, with comparatively reduced yet significant activity observed for carbon dots and ash. Although fresh powder extract exhibited the highest antidiabetic efficacy, carbon dots and ash also demonstrated appreciable inhibitory effects, possibly due to the presence of bioavailable minerals and altered surface chemistry. Overall, the findings suggest that *M. spicata* possesses substantial antioxidant and antidiabetic properties in vitro, irrespective of its form, with fresh powder being the most potent. Carbon dots offer added advantages of stability and tunability, making them promising alternatives. Further in vivo studies and clinical trials are warranted to validate the therapeutic potential of mint leaves for diabetes management and functional food applications.

Keywords: *Mentha spicata*, phytochemicals, antioxidant activity, antidiabetic activity, α-amylase inhibition, α-glucosidase inhibition, carbon dots, mint leaves

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease, characterized by alteration in the carbohydrate, protein and lipid metabolism. (Sola, 1995). India is the world's second most populous country, and having more people with type 2 diabetes mellitus, which is the major form of diabetes mellitus, accounting for 90% of cases worldwide (Sevugan et al., 2006). Postprandial hyperglycemia plays a vital role in development of type 2 diabetes mellitus and other complications caused by diabetes mellitus, such as retinopathic, nephropathic, neuropathic and cardiovascular complications (Zaman, 2006). One therapeutic approach suggested to reduce postprandial hyperglycemia is by the inhibition of two key enzymes linked to type 2 diabetes mellitus, namely α-glucosidase and α-amylase, in the digestive organs (Holman and Cull, 1999). Recently, it has been reported that compounds with combined antioxidant potential and antiglycation properties are effectively used to treat diabetes mellitus (Duraisamy et al., 2003).

Herbal drugs play an important part of traditional medicine and literature shows that more than 400 plant species having antidiabetic activity. Plants have been used for the treatment of diabetes since 1550 BC (Gray and Flatt, 1997) A number of spices and herbs have a long history of traditional use in treating elevated blood sugar levels (WHO, 1985). Plants which have antioxidant activity may show antidiabetic activity because oxidative stress may lead to diabetes mellitus. As *Mentha spicata* L. is already reported for antioxidant activity, hence, we selected this plant to assess its antidiabetic property.

The present study was undertaken to extend the survey of *M. spicata* L. for other medicinal properties than the ones reported so far. Hence, the ethanolic extract of leaves of *M. spicata* L. was used for its phytochemical analysis and evaluation of antioxidant potential through *in vitro* studies. Further inhibition of key enzymes linked to type 2 diabetes mellitus, i.e, α-amylase and α-glucosidase were also evaluated using ethanolic extract of *M. spicata* L. leaves- fresh powder, carbon (carbon dots) and ash. Hence, in the present study, fresh powder, carbon dots and ash prepared from mint leaves were utilized for evaluating their pharmacological efficacy.

FIGURE A: MINT (*MENTHA SPICATA*)

MATERIALS AND METHODS

Preparation of mint leaves extracts: Mint leaves were procured from the local area near Redhills, Chennai. Leaves were separately dried at room temperature in uncovered recipients, grounded to a fine powder and dried again. Extraction process, infusion, a type of maceration was utilized for the extract preparation. After grinding into fine powder, they were placed in a clean container. Then the extraction solvent 50% ethanol is then poured on top of the sample material, soaked, and kept for a short period of time (24h) to extract the bioactive constituents that are readily soluble. Then, the samples were filtered with grade 1 Whatman paper. 2% extracts were prepared using 50% ethanol; stored and utilized for further analysis. Pyrolysis procedure was utilized to prepare carbon dots from mint leaves and tested for antidiabetic efficacy. Simultaneously, mint ash was prepared and used.

Preliminary Phytochemical screening

The following protocol was followed for the preliminary phytochemical screening.

S.NO	EXPERIMENTS	OBSERVATIONS
1	**TEST FOR ALKALOIDS** **WAGNER‟S TEST** A few drops of Wagner‟s reagent are added to few ml of plant extract along the sides of test tube.	A reddish- Brown precipitate
2	**TEST FOR AMINO ACID** **NINHYDRIN TEST**	

	Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) are added to 2 ml of aqueous filtrate.	Appearance of purple colour
3	**TEST FOR CARBOHYDRATE** **MOLISCH' S TEST** To 2 ml of plant sample extract, two drops Molish' s reagent are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube **BENEDICT' S TEST** To 0.5 ml of filtrate, 0.5 ml of Benedict"s reagent is added. The mixture is heated on a boiling water bath for 2 minutes **FEHLING'S TEST** 1mL each of Fehling's solution A & B + 1mL filtrate + boiled in water bath	A violet ring A characteristic red/yellow/green colored precipitate A red precipitate
4	**TEST FOR PHENOLIC COMPOUNDS** **FERRIC CHLORIDE TEST** The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added	A dark green colour
5	**TEST FOR FLAVONOIDS** **ALKALINE REAGENT TEST** An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. **ACID TEST** Plant extract + conc. HCl	Yellow fluorescence Red color
6	**TEST FOR STEROLS** **SALKOWSKI'S TEST**	

	Filtrate + few drops of conc. H_2SO_4 (Shaken well and allowed to stand) (Ayoola *et al*., 2008).	Red colour (in lower layer)
7	**TEST FOR PROTEINS** The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatmann No. 1 filter paper and the filtrate is subjected to test for proteins **BIURET TEST** 2 ml of filtrate is treated with 1 drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets	Pink colour ethanolic layer
8	**TEST FOR GUM AND MUCILAGES** The extract (100 mg) is dissolved in 10 ml of distilled water and to this 2 ml of absolute alcohol is added with constant stirring.	White or cloudy precipitate
9	**TEST FOR DITERPENES** Plant extract is dissolved in distilled water + 3-4 drops of copper acetate solution	Emerald green colour
10	**TEST FOR TANNINS** **10% NaOH TEST** 0.4mL plant extract + 4mL 10% NaOH + shaken well	Formation of emulsion
12	**TEST FOR QUINONES** **CONC. HCL TEST** Plant extract + conc. HCl	A green colour
13	**TEST FOR GLYCOSIDES**	

	For 50 mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. **BORNTRAGER'S TEST** To 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammonia solution is added to it.	Pink colour

PHYTOCHEMICALS QUANTIFICATION

QUANTIFICATION OF ALKALOIDS

The extract was dissolved in 2 N of Hydrochloric acid (HCl) and then filtered. 1 ml of test extract solutions was transferred to separating funnel and added 5 ml of phosphate buffer pH 4.7 and 5 ml of bromocresol green (BCG) solution. The mixture was shaken and complex formed was extracted with 5 ml of chloroform. Chloroform layer was collected in 10 ml of volumetric flask and volume was made up to mark with chloroform. Absorbance was taken at 470 nm against blank. The alkaloid content was expressed in terms of mg of atrophine equivalents/ g of extract.

QUANTIFICATION OF TANNINS

The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution and diluted to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80, 100 µg/ ml) were prepared as described earlier. Absorbance for test and standard solutions were measured against the blank at 720 nm with an UV/ Visible spectrophotometer. The estimation of the tannin content was carried out in triplicate. The tannin content was expressed in terms of mg of gallic acid equivalents/ g of extract.

QUANTIFICATION OF TOTAL PHENOLS

The total phenolic contents in the extracts were measured using Folin-Ciocalteu reagent method (Makkar et al., 1993). Aqueous extract of 0.02 ml, 0.05 ml and 0.1 ml were transferred into test tubes and the volume was made up to 0.5 ml with distilled water. To this solution, 0.25 ml of Folin- Ciocalteu reagent (1N) and then 1.25 ml of 20% sodium carbonate was added, and the tubes were vortexed thoroughly. Absorbance was recorded at wavelength of 550 nm using UV- visible spectrophotometer after 40 minutes. The concentration of total phenolic compounds in the extract was calculated as Gallic acid equivalent (GAE) from standard curve. The total phenolic content was expressed as GAE/g of extract.

QUANTIFICATION OF TOTAL FLAVONOIDS

Total flavonoid content of the extracts was determined according to method of Nabavi et al. (2008). The aqueous plant extract (0.5 ml) was mixed with distilled water (2 ml) and subsequently with 5% $NaNO_2$ solution (0.15 ml). After 6 mins of incubation, 10% AlCl3 solution (0.15 ml) was added and then allowed to stand for 6 min, followed by addition of 1M NaOH solution (2 ml) to the mixture. Then, distilled water was added to the sample to make up the volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. The mixture s absorbance was determined at 510 nm using UV-Visible spectrophotometer. The test was performed in triplicate and the flavonoid content was expressed as mg of quercetin equivalent per g of extract (mg QE/g).

ASSAY OF ANTIOXIDANT ACTIVITY

ABTS RADICAL SCAVENGING ASSAY

Free radical scavenging activity of plant samples was determined by ABTS radical cation decolorization assay. ABTS·+ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 h

before use. ABTS·+ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of 5 µl of plant extract to 3.995 ml of diluted ABTS·+ solution, the absorbance was measured at 30 min after the initial mixing. An appropriate solvent blank was run in each assay. All the measurements were carried out at least three times. Percent inhibition of absorbance at 734 nm was calculated using the formula, ABTS·+ scavenging effect (%) = ((AB–AA)/ AB)×100 (2), where, AB is absorbance of ABTS radical + methanol; AA is absorbance of ABTS radical + sample extract/standard. Vitamin C was used as standard substance.

DPPH• RADICAL-SCAVENGING ACTIVITY

The DPPH• (2,2-Diphenyl-1-picrylhydrazyl (DPPH•) radical-scavenging activity was determined using the method proposed by Kim DO et al. (2003) with some modifications. The DPPH• is a blue-colored stable free radical, which is reduced to 2,2-diphenyl-1-picrylhydrazine (pale yellow), by reacting with an antioxidant. The DPPH• solution of $6x10^{-5}$ M was prepared in methanol. 3.4mL of this solution was subsequently added to 100µl of various concentrations of the samples. The samples were remained in dark for 45minutes and allowed to react at room temperature. The decrease in absorbance was measured at 517nm. The absorbance of the DPPH• radical without extract was also measured (A control). The % inhibition is calculated by the following formula:

% inhibition = $((A_{control} - A_{sample}) / A_{control})) * 100$ (A indicates absorbance)

FERRIC REDUCING ANTIOXIDANT POWER ASSAY:

The antioxidant capacity of the extract was estimated spectrophotometrically following the procedure of Benzie and Strain (Benzie and Strain, 1996). The method is based on the reduction of Fe^{3+} TPTZ complex (colorless complex) to Fe^{2+} -tripyridyltriazine (blue colored complex) formed by the action of electron donating antioxidants at low pH. This reaction is monitored by measuring the change in absorbance at 593 nm.

The Ferric reducing antioxidant power (FRAP) reagent was prepared by mixing 300 mM acetate buffer, 10 ml TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in the proportion of 10:1:1 at 37°. Freshly prepared

working FRAP reagent was pipetted using 1-5 ml variable micropipette (3.995 ml) and mixed with 5 µl of the appropriately diluted plant sample and mixed thoroughly. An intense blue color complex was formed when ferric tripyridyl triazine (Fe^{3+} TPTZ) complex was reduced to ferrous (Fe^{2+}) form and the absorbance at 593 nm was recorded against a reagent blank (3.995 ml FRAP reagent+5 µl distilled water) after 30 min incubation at 37°. All the determinations were performed in triplicates.

ASSESSMENT OF ANTIDIABETIC ACTIVITY

IN VITRO α -AMYLASE INHIBITION ACTIVITY

This assay was performed following the protocol described (Telagari and Hullatti 2015). The substrate solution (0.2 mL) containing starch and the pancreatic amylase solution (0.1 mL with a concentration of 2 units/mL) were added to sample solution (0.2 mL) at different concentrations ranging from 1000 µg/mL to 3000 µg/mL.

The reaction took ten minutes to finish at 37 °C, before being stopped by adding 0.5 mL of 50% acetic acid to each tube. The tubes were centrifuged for 5 min at 4 °C at 3000× rpm, and the optic density of the supernatant was measured using a spectrophotometer at 595 nm. Acarbose (α-amylase inhibitor) was utilized as a positive control in this test. For each concentration, the experiment was performed three times.

The inhibitory activity of the enzyme was determined using the formula:

$$\text{Activity inhibition} = [(A - B)/A] \times 100$$

where A is the control absorbance and B is the sample absorbance.

IN VITRO α-GLUCOSIDASE INHIBITION ACTIVITY

This assay was performed following the protocol described (Telagari and Hullatti, 2015). A total of 50 µL of samples, at different concentrations ranging from 1000 to 3000 µg/mL, was prepared and incubated with 10 µL of α-glucosidase (maltase at a concentration of 1 unit/mL) and 125 µL of 0.1 M phosphate buffer for 20 min at 37 °C. 20 µL the substrate (pNPG) at 1 M was prepared and added to

the preparation and the whole was incubated for 30 min. A total of 50 µL of Na_2CO_3 (0.1 N) was added to stop the reaction. The optical density measurements were carried out at 405 nm. Acarbose was used as the positive control and the experiment was replicated three times for each concentration.

The inhibitory activity of the enzyme was determined using the formula:

Activity inhibition = [(A − B)/A] × 100

where A is the control's absorbance and B is the sample's absorbance.

STATISTICAL ANALYSIS

In this study, all the experiments were carried out in three independent biological replicates, and data were reported as mean ± standard deviation for each set of conditions. Statistical significance of the data was tested through one - way analysis of variance (ANOVA) using least significant difference with $p<0.05$, $p<0.01$, $p<0.001$.

RESULTS

TABLE 1. PRELIMINARY PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF LEAVES OF *MENTHA SPICATA*

S.NO	EXPERIMENTS	INFERENCES
1	Test for Alkaloids	PRESENT
2	Test for Amino acids	ABSENT
3	Test for Carbohydrates - Molisch's Test	PRESENT
4	Test for Carbohydrates - Benedict's Test	PRESENT
5	Test for Carbohydrates - Fehling's Test	PRESENT
6	Test for Phenols	PRESENT
7	Test for Flavonoids - alkaline reagent test	PRESENT
8	Test for Sterols	PRESENT
9	Test for Proteins	ABSENT
10	Test for Diterpenoids	PRESENT
11	Test for Gum and mucilages	ABSENT
12	Test for Tannins	ABSENT
13	Test for Quinones	PRESENT
14	Test for Glycosides	ABSENT

Table 1 represents the phytochemical screening of ethanolic extracts of mint leaves. It showed the presence of alkaloids, carbohydrates, phenols, flavonoids, sterols, diterpenoids and quinones.

FIGURE 1. QUANTIFICATION OF PHYTOCHEMICALS IN ETHANOLIC EXTRACT OF LEAVES OF *MENTHA SPICATA.* (N=5; Values are Mean ± SD)

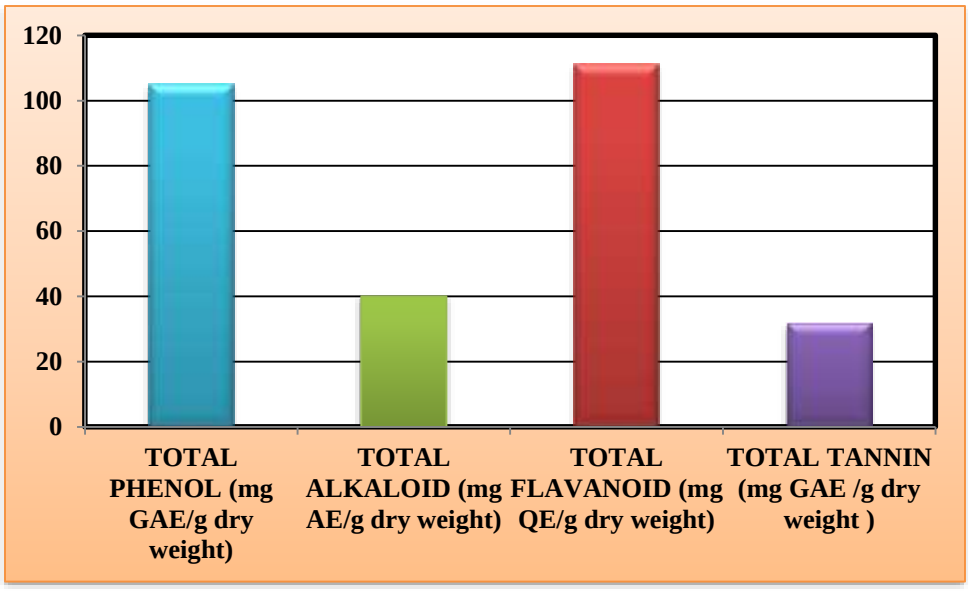
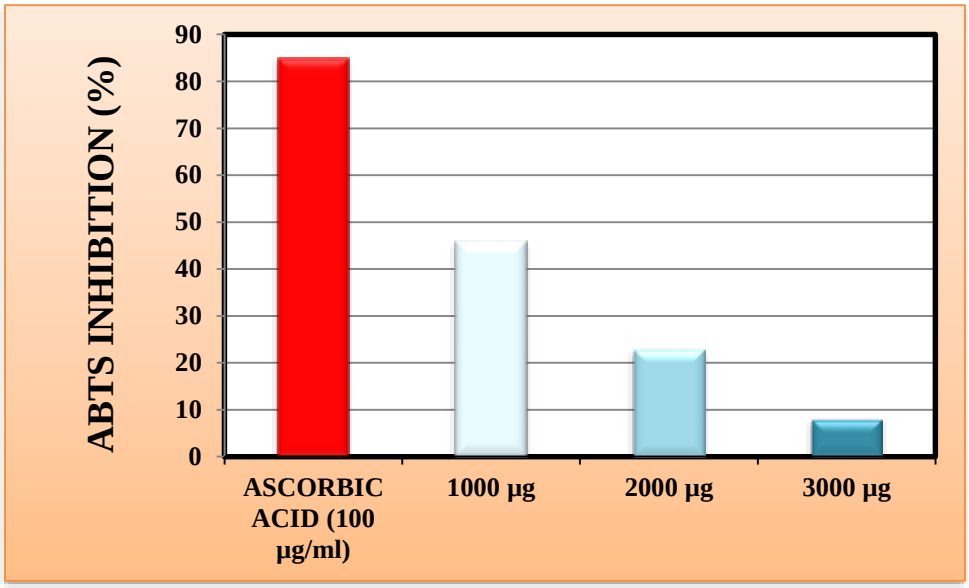


Figure 1 depicts the varying concentration of alkaloids, tannins, flavonoids and phenols in ethanolic extract of mint leaves. It shows the increased concentration of phenols and flavonoids compared to alkaloids and tannins.

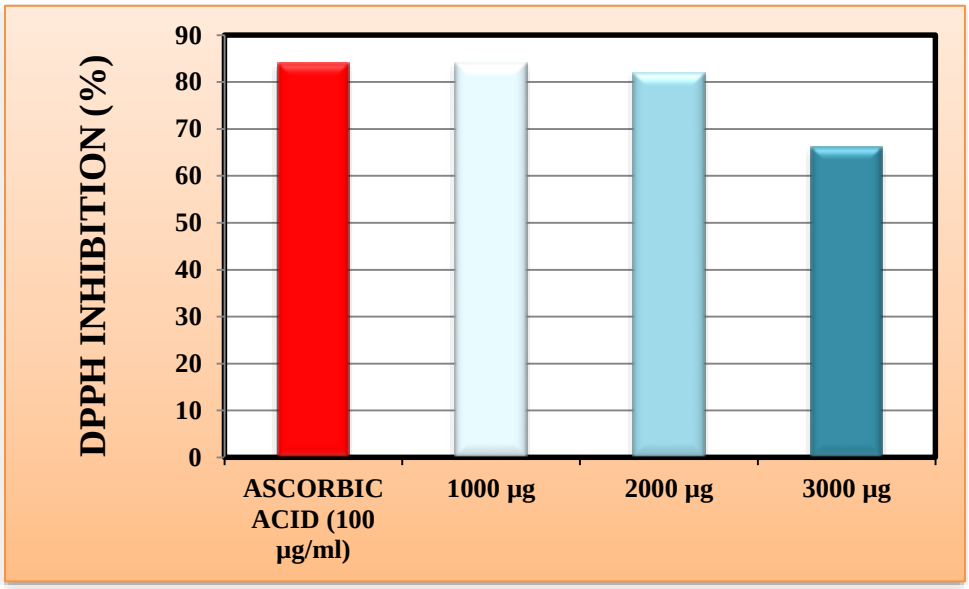
FIGURE 2: ANTI-OXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF MINT LEAVES.

(N=5)

i. 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonate (ABTS) inhibition assay



ii. 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition assay



iii.FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

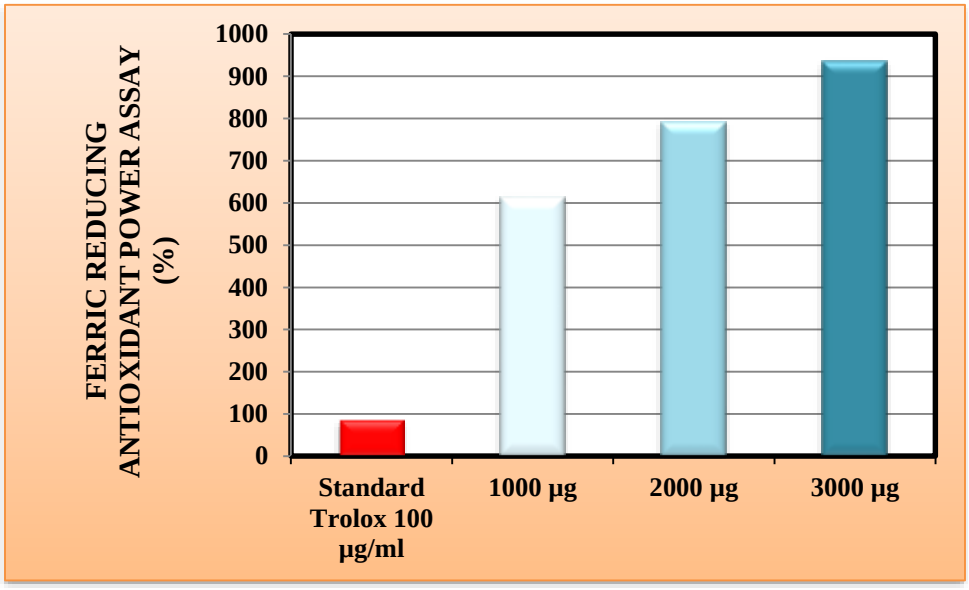


Figure 2 (i, ii and iii) reveals the gradual decrease in ABTS and DPPH radical inhibition activity on increasing the concentration, while increase in nitric oxide scavenging activity on increasing the concentration**ASSESSMENT OF ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF FRESH POWDER, CARBON DOTS AND ASH PREPARED FROM MINT LEAVES (N=5)**

FIGURE 3. IN VITRO α -AMYLASE INHIBITION ACTIVITY

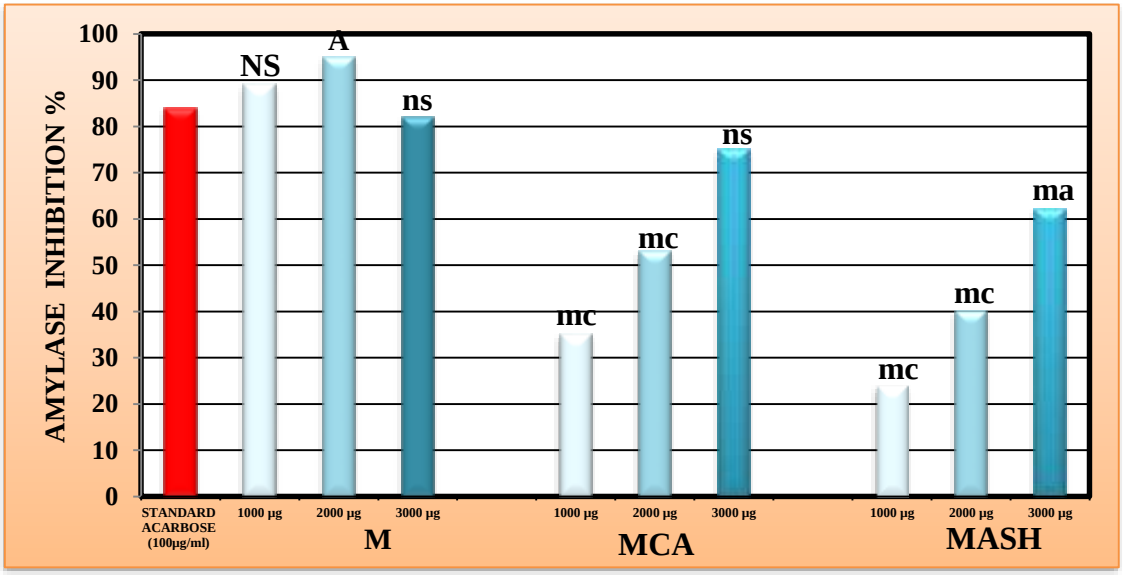
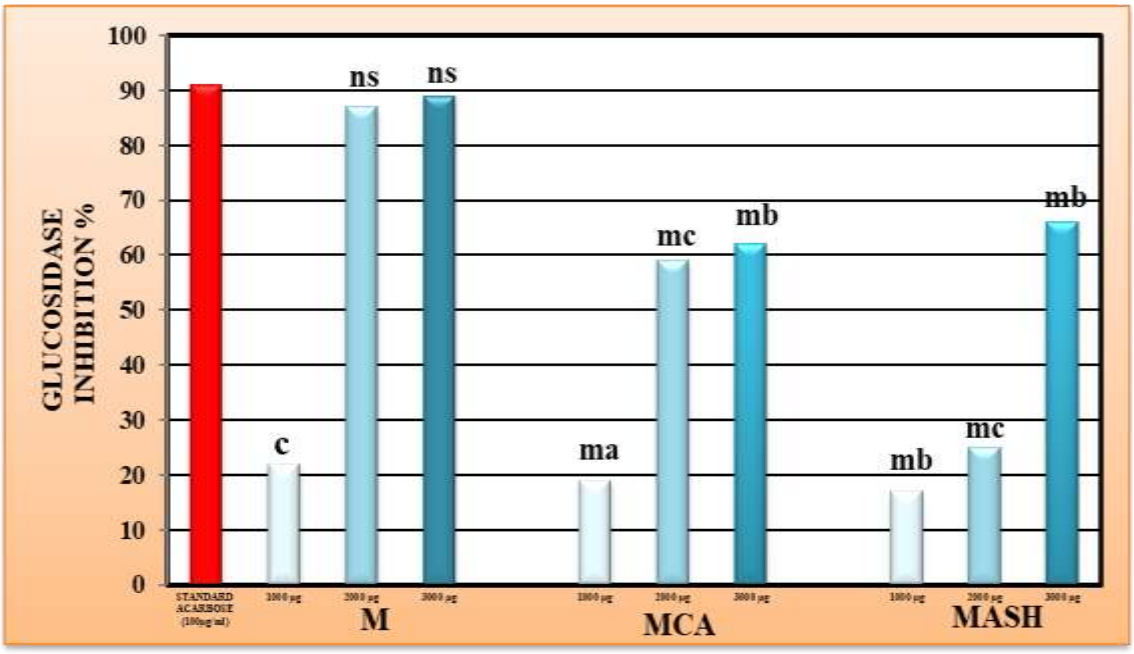


FIGURE 4. IN VITRO α-GLUCOSIDASE INHIBITION ACTIVITY



M: ETHANOLIC EXTRACT OF FRESH POWDER OF MINT LEAVES

MCA: ETHANOLIC EXTRACT OF CARBON DOTS OF MINT LEAVES

MASH: ETHANOLIC EXTRACT OF ASH OF MINT LEAVES

ns: not significant, A/a: $p<0.05$; B/b: $p<0.01$; C/C: $p<0.001$

A/B/C: Increase, a/b/c: Decrease

m: comparison with ethanolic extract of fresh powder of mint leaves

Figure 3 depicts the increase in α amylase inhibition by the ethanolic extract of fresh powder of mint leaves on increasing the concentration from 1000µg to 2000µg, while on increasing the concentration to 3000µg, decline in α amylase inhibition was observed. In contrast, gradual increase in α amylase inhibition activity was observed for both carbon dots and ash on increasing the concentration. However, compared to fresh powder extract, α amylase inhibition activity was observed to be decreased for both carbon dots and ash.

Figure 4 depicts the increase in α glucosidase inhibition by the ethanolic extract of fresh powder of mint leaves on increasing the concentration from 1000µg to 3000µg. Similarly, gradual increase in α glucosidase inhibition activity was observed for both carbon dots and ash on increasing the concentration. However, compared to fresh powder extract, α glucosidase inhibition activity was observed to be decreased for both carbon dots and ash.

DISCUSSION

Present study ascertained the preliminary phytochemical screening, phytochemical quantification and antioxidant activity of ethanolic extract of fresh powder of mint leaves. In addition, antidiabetic activity was evaluated for ethanolic extract of fresh powder, carbon dots and ash of mint leaves. Table 1 represents the phytochemical screening of ethanolic extracts of mint leaves. It showed the presence of alkaloids, carbohydrates, phenols, flavonoids, sterols, diterpenoids and quinones. Similarly, EL-Haoud and coresearchers (2018) demonstrated a broad spectrum of bioactive compounds from preliminary screening of *M. spicata*; revealed the presence of polyphenols, flavonoids, tannins, sterols, triterpenes, and glycosides. In addition, another study by Mikaili and coresearchers (2013) investigated the qualitative analysis of phytochemical compounds of various extracts of leaves, stem, and root of *M. arvensis*. Findings showed the presence of different phytochemical compounds, such as alkaloids and flavonoids polyphenols, tannins, cardiac glycosides, indicating their distribution in the whole plant.

In the context of quantification of phytochemicals, alkaloids, tannins, flavonoids and phenols were quantified using the respective protocols. Figure 1 depicts the varying concentration of alkaloids, tannins, flavonoids and phenols in ethanolic extract of mint leaves. It portrays the increased concentration of phenols and flavonoids compared to alkaloids and tannins. Similarly, Mikaili and coresearchers (2013) reported that stem extract found to contain more alkaloids compared to leaves and root, and; root found to contain more tannins compared to leaves and stem, which substantiate our study results. In addition, in the same study, it was described that leaves contain high proportion of phenols and flavonoids compared to stem and root, which fits our study results.

Figure 2 (i, ii and iii) reveals the gradual decrease in ABTS and DPPH radical inhibition activity on increasing the concentration, while increase in nitric oxide scavenging activity on increasing the concentration. It highlights that the presence of phytochemicals, in particular phenols and flavonoids may be attributed to the significant antioxidant activity towards DPPH, ABTS and ferric ion radicals. Regarding *M. spicata*, many studies have evaluated its antioxidant activity either by measuring its

effectiveness in scavenging free radicals or by directly assaying the products formed using photometric techniques (Hussain et al., 2010; Getahun et al., 2008; Nickavar et al., 2008).

Moreover, the antidiabetic potential was determined for mint leaves in all three forms (fresh powder, carbon and ash). In the context of glycemic control, components which are decreasing blood glucose absorption are mostly considered as anti-diabetic. In contrast, foods which are decreasing the raise in blood glucose level can also be taken into account. Considering this, key dietary carbohydrate metabolizing enzymes inhibition (α amylase and α glucosidase) were tested on addition of ethanolic extract of mint leaves (fresh powder, carbon and ash). Inhibition of these enzymes will be diminishing the blood glucose level raise due to their restricted activity towards dietary carbohydrates metabolism.

Figure 3 depicts the increase in α amylase inhibition by the ethanolic extract of fresh powder of mint leaves on increasing the concentration from 1000µg to 2000µg, while on increasing the concentration to 3000µg, decline in α amylase inhibition was observed. In contrast, gradual increase in α amylase inhibition activity was observed for both carbon dots and ash on increasing the concentration. However, compared to fresh powder extract, α amylase inhibition activity was observed to be decreased for both carbon dots and ash.

Similarly, Figure 4 depicts the increase in α glucosidase inhibition by the ethanolic extract of fresh powder of mint leaves on increasing the concentration from 1000µg to 3000µg. Similarly, gradual increase in α glucosidase inhibition activity was observed for both carbon dots and ash on increasing the concentration. However, compared to fresh powder extract, α glucosidase inhibition activity was observed to be decreased for both carbon dots and ash.

Concomitantly, a very recent investigation tested this powder on two carbohydrate hydrolyzing enzymes, namely, α-amylase and α-glucosidase (Bouyahya et al., 2020). In fact, inhibiting these two enzymes prevents the digestion of carbohydrates, which is a promising strategy in the treatment of diabetes. The results of the study showed that the leaf essential oil of this herb at doses of 200 and 250 μL was able to inhibit α-amylase and α-glucosidase respectively. However, the antidiabetic activity was not tested for mint leaves' carbon and ash. Hence, our study focussed to explore the antidiabetic

potential of both carbon dots and ash. It is well known that mint serves as an important source of macro and micrometallic elements, which are essential for human health. Coherently, significant antidiabetic efficacy was noted for carbon dots and ash of mint leaves. However, both were found to exhibit decreased antidiabetic potential compared to fresh powder extract. Still, they were found to mediate antidiabetic potential near to that of fresh powder extract of mint leaves.

Since, carbon dot preparation is often considered better than fresh extract preparation because it offers advantages like greater stability, tunable properties, and potential for targeted applications due to its smaller size and well-defined surface chemistry. Fresh extracts are less reliable, perishable, hence carbon dots were prepared and simultaneously ash was also prepared to understand and explore the antidiabetic activity of fresh, carbon and ash form of mint leaves.

From these studies, it can be inferred that *M. spicata* may be used as an antidiabetic agent; however, further investigations, as well as clinical trials, must be carried out to evaluate this benefit in humans.

CONCLUSION

Present study reveals that the ethanolic extract of fresh powder, carbon dots and ash were found to possess strong antidiabetic properties in vitro by displaying significant inhibition of key regulatory enzymes of glucose level α amylase and α glucosidase. This was also reflected in their antioxidant activity revealed from ABTS, DPPH and ferric ion scavenging activities. The compounds responsible for these activities may include both phenols and flavonoids, found to be high in ethanolic extract of fresh powder of mint leaves. Similarly, minerals present in the carbon-dots and ash may be responsible for significant antidiabetic activity depicted from inhibition of key regulatory enzymes of glucose level α amylase and α glucosidase. Overall, this study supports the notion that mint leaves either fresh, carbon or ash form may lend themselves for use in food formulations and as potential health promoting ingredients.

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