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Research Article

Effect of Selected Plant Extracts on Pancreatic Lipase Inhibition, Pancreatic Cholesterol Esterase Activities and Cholesterol Micellization

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Abstract

The study shows that the four edible plant extracts (Dendrobium Nobile, Hawthorn, Wolfberry and Tea Extract) consist of a wide range of functional factors. The total polysaccharide content in the plant extract ranged from 6.17% to 25.2%. The plant extracts revealed positive hypolipidemic effects for the index parameters such as pancreatic lipase inhibition, cholesterol esterase inhibitory activities and cholesterol micellization solubility. The extracts had a pancreatic lipase inhibition rate between 27 to 41% and the level of inhibition of cholesterol esterase depends on the different types of plant extracts varied from 19 to 47%. The extracts (12 mg/mL) significantly inhibited cholesterol micelle formation from about 19 to 42%. The findings of the study were compared to other studies on similar extracts and showed potential to delay the digestion and absorption of fat.

Keywords: Edible plant extracts, functional factors, hypolipidemic effects, cholesterol micellization.

Introduction

Hyperlipidemia is characterized by elevated level of cholesterol and triglycerides in plasma and leads to health risks (Su *et al.*, 2016). Toma *et al.* (2014) reported that long-term hyperlipidemia was an important factor contributing to the progression of micro and macro-vascular complications, including microangiopathy, cardiovascular, cerebrovascular and metabolic syndrome diseases. During the last two decades, the prevalence of hyperlipidemia has been a focal point of different researchers due to modern lifestyle and increase consumption of a fat diet (Jacobson *et al.*, 2007). Birari *et al.* (2007) reported that the new attempt to reduce the absorption of free fatty acids is by delaying triglyceride digestion with the inhibition of pancreatic lipase. Pancreatic cholesterol esterase plays a crucial role in hydrolyzing of dietary cholesterol esters (Brodt *et al.*, 1995). Furthermore, the principal steps in the absorption of dietary cholesterol are emulsification, hydrolysis of the ester bond by a pancreatic esterase, micellar solubilization and absorption in the proximal jejunum (Hui *et al.*, 2005). In addition, the inhibition of these enzymes could help in reducing energy value of food, by reducing its availability and extension of the digestion process, thereby reducing the body weight and causing far-reaching health benefits (Satouchi *et al.*, 1974; Han *et al.*, 2001). During the last three decades, Orlistat, a specific drug for inhibiting pancreatic lipase that reduces dietary fat absorption by 30%, has been approved for clinical use (Satouchi *et al.*, 1974; Hill *et al.*, 1999). However, Orlistat showed adverse side effects, such as fecal incontinence, flatulence, and steatorrhea (Weigle, 2003; Birari *et al.*, 2007). In recent years, researchers have turned their attention on the use of botanical materials as potential source of new drugs or as the source of the main active compounds for new medicaments (Gullo *et al.*, 2006). Natural products prepared from traditional medicinal plants and microbial sources have always presented an exciting opportunity for the development of new therapeutic agents. An analysis of the origin of the drugs launched in the last twenty-five years showed that about half of all compounds that were successful in clinical trials have been derived from a natural origin (Newman *et al.*, 2007). Natural products provide a wide variety of pancreatic lipase inhibitors that can possibly be developed into clinical products. In recent years, polysaccharides from food plants have emerged as an important class of bioactive natural products that are being widely studied in order to better understand the relationship between physico-chemical

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properties and biological activities of these compounds (Inngjerdingen *et al.,* 2005; Gross *et al.*, 2006; Chen *et al.*, 2008). On one side, Xing *et al.* (2014) identified O-acetylated glucomannan (Dendronan) as the major polysaccharide that might play a great role in the medicinal properties of *Dendrobium offinale*. On the other side, Wang *et al.* (2012) studied the antioxidant activities potential of tea polysaccharide fractions obtained by ultrafiltration and reported that the tea polysaccharide fraction 1 (TPS1) with a molecular weight of 2.40×10^5 Da and crude tea polysaccharide (CTPS) had stronger antioxidant activity compared with other fraction with lower molecular weight. Moreover, Kirakosyan *et al.* (2003) studied the effect of drought and cold stress treatment on the antioxidant capacity of polyphenolic extracts from Leaves of *Crataegus laevigata* and *Crataegus monogyna* (Hawthorn) and concluded that these kinds of stress treatments can enhance the levels of important secondary metabolites and their total antioxidant capacities in leaves of *Crataegus*. Furthermore, Wolfberry is the common name of the fruits *Lycium barbarum* or *Lycium chinense,* which are two very closely related species. It belongs to the family Solanaceae and it can be found in many regions of the world. Polysaccharides from wolfberry are reported to possess important bioactive functions, including hypoglycemic and hypolipidemic activities (Luo *et al.,* 1999) and immuno-modulating action (Luo *et al.,* 2004). However, the potential of developing successful natural products for the management of obesity is still largely unexplored. The screening and optimization of safe and effective lipid lowering phytochemicals would provide an excellent new strategy in combating obesity and its complications (Birari *et al.*, 2007). Therefore, the purpose of this study was to compare the effect of different plant material extracts on the inhibition of pancreatic lipase and cholesterol esterase. We have experimented four edible plants (Dendrobium Nobile Lindl, Hawthorn, Chinese wolfberry and Tea extract) to study their effects on the inhibition of pancreatic lipase and pancreatic cholesterol esterase activities, as well as inhibition of cholesterol micellization.

Materials and methods

Plant extracts: Dendrobium nobile extract, Wolfberry extract and Tea extract were purchased from Nanjing Zelang Pharmaceutical Technology Co., Ltd.; Hawthorn extract was purchased from Zhejiang grid Pharmaceutical Co., Ltd; Ethyl maltol was purchased from Love spice Group Co., Ltd.; stevia leaves sugar, Sodium taurocholate, Orlistat, Simvastatin Gallic acid, Enzyme and cholesterol kit were purchased by the Nanjing Zelang Pharmaceutical Technology Co., Ltd. Other used chemicals were food grade and purchased from Sinopharm Group Co., Ltd., analytically pure (Check on ethanol bottle).

Determination of Molecular weight distribution (MW): The molecular weight distribution was determined using high performance gel permeation chromatography (HPGPC) according to the method of (Li *et al.,* 2018). MW determination was performed on a Shimadzu LC-20AT chromatography equipped with Shodex OH pak SB-803 column (8×300mm i.d., YMC Co. Ltd, Kyoto, Japan) or Shodex OH pak SB-804 column (8×300mm i.d., YMC Co. Ltd, Kyoto, Japan) with refractive index detector (Shimadzu RID-10 A).

Determination of polysaccharide content: The total amount of polysaccharide in the raw material was measured using the Phenol Sulphuric Acid Method (Dubois *et al.,* 1956). About 0.3 g of test powder was precisely weighed, placed in 250 mL round bottom flask and 200 mL of distilled water was added to it. The solution was then heated at 95°C for 2 h, cooled and transferred into 250 mL capacity flask. The volume was made up then the mixture was shaken and filtered. Two milliliters of filtrate were then transferred to a 15 mL centrifuge tube and 10 mL of absolute ethanol were added, shaken and refrigerated for 1 h at 4°C. After the mixture was centrifuged at 4000 rpm/min for 20 min. The supernatant was discarded, and the precipitates were washed twice with 8 mL of 80% ethanol solution. Upon discarding the supernatant, the precipitate was dissolved with hot water, cooled and transferred to 25 mL volumetric flask and made up to the appropriate volume. The polysaccharide values were then measured by a five-point standard calibration curve using UV-visible spectrophotometry (Indicate the type of UV used and from where) at 488 nm wavelength.

Determination of total flavonoids: The total flavonoid content of extracts Dendrobium, Hawthorn, Wolfberry and Tea extract was measured against a rutin standard solution curve using UV-visible spectrophotometry (type and origin) at a 415 nm wavelength. About 1 g of plant extract powder was weighed and extracted in the Soxhlet extractor according to the aluminum chloride colorimeter method described by (Hsieh *et al.*, 2014). The extracts were evaporated to dryness and the residues were diluted in 50 mL ethanol with the concentration of 60%. Take as the test stock solution, filter the precise amount of filtrate 5 mL, 25 mL volumetric flask, add water to the mark, and shake well. Measure the precise amount of 2 mL, 25 mL volumetric flasks according to the standard curve preparation method, add water to make 6 mL according to the determination of absorbance, read the weight of rutin in the test solution from the standard curve.

Determination of total polyphenol: The amount of total phenolics contents in extracts of Dendrobium, Wolfberry, Hawthorn and Tea extract was determined according to the

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method described by (Singleton *et al.*, 1965). Briefly, the diluted sample (1.0 mL) was added to Folin–Ciocalteu reagent (5.0 mL, diluted 10-fold in deionised water). After 5 min, 4.0 mL of saturated sodium carbonate solution about 75 g/L was added. The mixture was thoroughly mixed, incubation for 1 h at room temperature and the absorbance was measured at 765 nm and measurements were made in triplicate.

Determination of pancreatic lipase (PL) inhibition: Inhibition of pancreatic lipase of four extracts was spectrophotometrically performed according to previous method with slight modification (Lewis and Liu, 2012). The extracts were incubated with mixtures containing 5 mM deoxytaurocholic acid, 0.2 mM p-nitrophenyl palmitate (p-NPP) in 50 mM sodium phosphate monobasic buffer, pH 8.0. The reaction was then initiated by addition of porcine pancreatic lipase (10 mg/mL). After incubation for 5 min at 37°C, the mixtures were measured at absorbance of 410 nm. Orlistat was used as a positive control. The inhibition was calculated according to the following formula:
Inhibition (%) = (Abs Control-Abs Sample)/Abs Control × 100,

Where the Abs control was the absorbance without a sample, the Abs samples was the absorbance of the sample extract.

Determination of cholesterol Esterase Inhibition (CEase): The cholesterol inhibition assay was performed according to previous method described by Hosie *et al.* (1987) with some modifications. The temperature was maintained at 25±0.2°C. p-nitrophenylbutyrate (pNPB) was dissolved in acetonitrile. The final concentration of acetonitrile in the test solution was 2%. First, CEase (0.18 µL, 200 µL) were pre-incubated with different concentrations of inhibitor in 0.1 M sodium phosphate buffer (pH 7.04, containing 0.1 M NaCl) for 5 min. Then, the pNPB (0.1 mM) was added to the reaction mixture and the enzyme reaction was monitored for 1 min by measuring the change in absorbance at 405 nm. The experiment was performed in triplicate.

Determination of cholesterol micellization solubility: The effects of the four plant material extracts on the micellar solubility of cholesterol were examined according to the method of (Zhang *et al.*, 2012). A bile salt micellar solution (1 mL) containing 0.4 mM cholesterol, 10 mM sodium taurocholate, 1 mM oleic acid, 132 mM NaCl and 15 mM sodium phosphate at pH 6.8 was prepared by sonication. These micellar suspensions are kept at 37°C for 24 h. Various amounts of plant extracts are added into the 5 mL micellar solution. The micellar solution without plant extracts is used as a substrate blank to calculate the recovery. Then the mixtures are incubated at 37°C for 2 h shaker bath. Mixtures are then centrifuged at 16,000 rpm for 20 min at 37°C. The incorporated cholesterol in the supernatants, which represented micellar cholesterol, was obtained after ultracentrifugation. The supernatant was collected and then the concentration of cholesterol in the micelles was determined enzymatically. Each measurement was repeated three times.

Data analysis: An analysis of variance (ANOVA) was applied and $p \leq 0.05$ was considered to indicate statistical significance. Statistical data analysis was undertaken using SPSS Statistics 20.

Results and discussion

Molecular weight distribution: Dendrobium, wolfberry, Hawthorn and Tea are herbaceous plant highly valued in Chinese medicine and archived in the Chinese literature (Gan *et al.*, 2004; Hsieh *et al.*, 2008; Li *et al.*, 2007; Redgwell *et al.*, 2011). The distribution of molecular weight (MW), Dendrobium, Wolfberry, Hawthorn and Tea Extracts were 62000 Da, 45000Da, 1200Da and 900Da respectively the sample extracts were qualitatively analyzed to determine their molecular weight distribution. As shown in Table 1, the result of the extracts showed that the extract of Dendrobium and Wolfberry had more molecular weight than the other two extracts this difference can be due to the nature of the plants extracts. Wei *et al.* (2016) characterized the structure and immunomodulation effects of polysaccharides isolated from *Dendrobium officinale* and found the two isolated fractions have average molecular weight of 7.3×10^5, 8.1×10^5, and 6.7×10^5 Da respectively. Fan *et al.* (2010) studied the composition and antioxidant activity of polysaccharides from wolfberry, cherry, kiwi and cranberry fruits and reported that the MW of raw extracts from wolfberry were 604.5 kDa and 57.6 kDa, respectively. Wei *et al.* (2010) reported that tea flower polysaccharides obtained by traditional water extraction mainly consisted of two kinds of polysaccharides with the molecular weight of 31 kDa and 5000 Da. In addition, Wang *et al.* (2012) studied the antioxidant activity of three tea polysaccharide fraction and found that the molecular weights of three fractions were around 2.40×10^5Da, 2.14×10^4Da, and 2.46×10^3Da, respectively.

Table 1. Molecular Weight distribution of Dendrobium Nobile, wolfberry extracts, Hawthorn extract and Tea extract using high performance gel permeation chromatography (HPGPC).

Plant extracts	Molecular Weight Distribution (%)			
	40-65 KDa	3-4 KDa	1-2 KDa	0.1-0.5 KDa
Dendrobium	53.02	-	26.78	20.21
wolfberry	50.88	-	28.41	20.71
Hawthorn	-	97.08	-	2.92
Tea	16.21	-	45.44	38.35

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Total polysaccharides: Varieties of plants, algae and edible fungi have been widely used to evaluate the hypolipidemic effect of natural compounds, especially polysaccharides (Ducluzeau *et al.*, 2003; Nakagawa, 2009; Wang *et al.*, 2013). Plants are also containing abundant polysaccharide that are useful in foods and medications and several studies showed the beneficial functions of polysaccharides, such as strengthening anti-inflammatory, anti-tumor and innate properties (Liao *et al.*, 2011; Wang *et al.*, 2013). As shown in Table 2, the results reveals that the total polysaccharides content were high Dendrobium extract (25.2%) followed by Wolfberry extract (20.9%) while, Howthorn extract (11.3%) and Tea extract (6.17%) showed the lowest polysaccharide content. Deng *et al.* (2018) reported that the total polysaccharide content in *Dendrobium devonianum* extracts were 28.3% while Redgwell *et al.* (2011) reported 17% in a Chinese Wolfberry extract (*Lycium barbarum*). On the other side, Wei *et al.* (2010) studied the effect of different extraction methods on the tea flower total polysaccharide content and reported that traditional water extraction was found to be the optimal method with highest yield of tea flower polysaccharides and highest neutral and acid saccharides contents in tea flower polysaccharides.

Table 2. Total Polysaccharides in extracts Dendrobium, wolfberry, Hawthorn and Tea.

Plant extract	Mean ±SD (mg/g)
Hawthorn	11.3±0.62
Tea	6.17±0.51
Wolfberry	20.9±0.98
Dendrobium	25.2±1.22

Total polyphenol: Polyphenols from tea are generally useful in tea beverages to reduce hyperlipidemia (Mizukami *et al.*, 2007; Yang *et al.*, 2007; Rusak *et al.*, 2008). As shown in Table 3, the total polyphenol content was significantly different in the four extracts. The tea extract showed more polyphenolic compound (115.3 mg/g) followed by hawthorn extract (69.8 mg/g), wolfberry extract (47.6 mg/g) and Dendrobium extract (31.5 mg/g). Turkmen *et al.* (2006) reported about 1.8 to 99.8 mg/g total polyphenols in different black tea extracts. Moretti *et al.* (2013) reported that the percentage of total polyphenols in *Dendrobium speciosum* stems and leaves was around 1.15% and 1.06%, respectively. In addition, Klongkumnuankarn *et al.* (2015) reported that the total phenolic content of the dried plant material was 1.13 mg GAE/g. Bahorun *et al.* (2003) reported that the total polyphenol in Howthorn extracts increased with increasing the maximum growth of Howthorn plants between 30-35 d and reached 47.40 mg/g dry weight.

Table 3. The total phenolic in extracts Hawthorn, Tea, wolfberry and Dendrobium.

Plant extract	Mean ±SD (mg/g)
Hawthorn	69.8±0.77
Tea	115.3±1.39
Wolfberry	47.6±0.52
Dendrobium	31.5±0.34

Total flavonoids: Medicinal plants are known for their powerful antioxidant properties, as they contain bioactive compounds such as carotenoids, benzoic acid, cinnamic acid, folic acid, phenols and flavonoids (Amro *et al.*, 2002). Flavonoids are plant secondary metabolites widely distributed in the plant kingdom and more than 6000 flavonoids have been identified (Khatiwora *et al.*, 2010). As shown in Table 4, the total flavonoid content was significantly different in the four extracts ($P \leq 0.05$). Hawthorn extracts showed higher flavonoids content (5.41 mg/g) followed by tea extract (2.37 mg/g), wolfberry extract (1.26 mg/g) and Dendrobium extract (1.13 mg/g). Pan *et al.* (2012) studied the optimization of flavonoids compounds from hawthorn seed using ultrasound-assisted extraction and found that the total flavonoids content in hawthorn seeds extract was 16.45 mg/g, which was 1.32-fold the yield of conventional reflux extraction. Istrati *et al.* (2013) reported that the total flavonoid content in wolfberry fruit was 53.06 mg QE/100g. Moretti *et al.* (2013) found that the percentage of total flavonoids of methanolic Dendrobium stems and leaves extracts were 0.21% and 0.12% respectively. The variation total flavonoids content from respective plant extracts compared to previous studies might be attributed to the extraction methods.

Table 4. The total flavonoid in extracts Hawthorn, Tea, wolfberry and Dendrobium.

Plant extract	Mean ±SD (mg/g)
Hawthorn	5.41±0.24
Tea	2.37±0.21
Wolfberry	1.26±0.20
Dendrobium	1.13±0.20

Pancreatic lipase (PL) inhibition: Pancreatic lipase is an enzyme responsible for fat metabolism thus its inhibition can significantly reduce fat absorption and the risk of obesity. As shown in Fig. 1, the four plant extracts showed significant inhibition capacity in range of 27-41% on pancreatic lipase (how about the control). Tea extract showed the lowest inhibition capacity (27.68%) compared to Dendrobium extracts (41.50%), Wolfberry (38.05%) and Hawthorn (32.20%).

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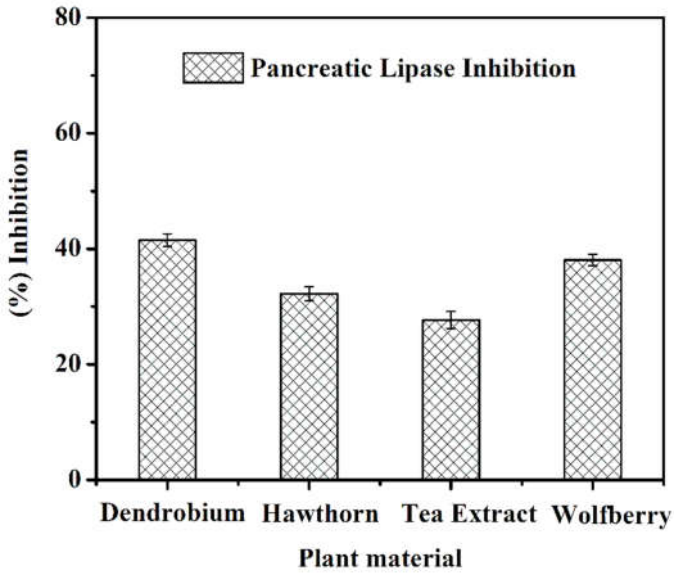
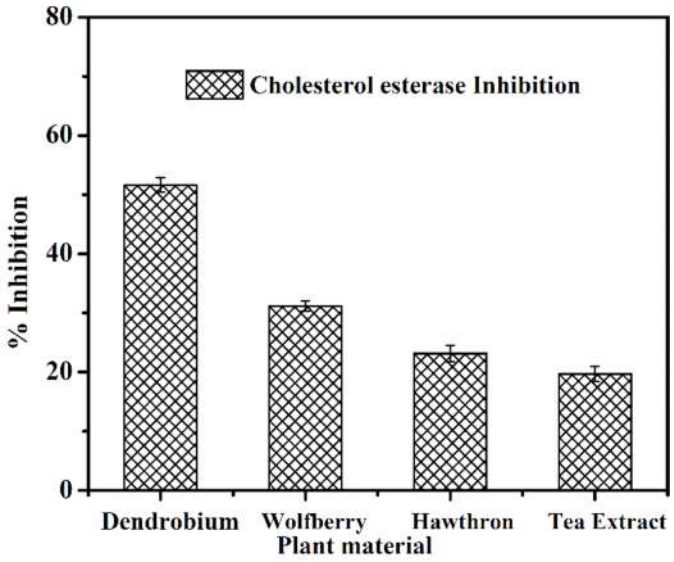
Nakai *et al.* (2005) studied inhibitory effects of oolong tea polyphenols on pancreatic lipase in vitro; and suggested that the presence of galloyl moieties within their chemical structures and/or the polymerization of flavan-3-ols in oolong tea were required for enhancement of pancreatic lipase inhibition. In addition Yuda *et al.* (2012) studied the inhibition of pancreatic lipase of polyphenols extracted from Black Tea (*Camellia sinensis*) in vitro and found that all polyphenols extracts inhibited pancreatic lipase but extracts obtained at 100 to 140°C showed the greatest lipase inhibition (IC50s of 0.9 to 1.3 µg/mL), consistent with the optimal extraction of the aflavins and catechins except catechin by HCW between 130 and 150°C. Inthongkaew *et al.* (2017) studied the pancreatic lipase inhibition stimulatory effect of phenolic compounds from *Dendrobium formosum* and found that 5-methoxy-7-hydroxy-9,10-dihydro-1,4-phenanthrenequinone has the highest pancreatic lipase inhibitory effects with an IC50 value of 69.45±10.14 µM. On the other side, Zhao *et al.* (2018), studied the effect of black wolfberry (*Lycium ruthenicum Murr.*) seed oil on the pancreatic lipase inhibition and found that the black wolfberry seed oil exhibited strong pancreatic lipase inhibition activity with IC_{50} 2.63 mg/mL. The lipase inhibition effect could be attributed to the presence of phenolic, flavonoids compounds and molecular weight distribution of the extracts.

Fig. 1. Effect of all four plants material on pancreatic lipase inhibition, all the PL inhibition values measured in the four plant extracts were significantly different from each other ($p<0.05$).



Cholesterol esterase inhibition: The Cholesterol esterase enzyme (CEase) hydrolyzes cholesterol esters to cholesterol and free fatty acid prior to their absorption. Since, intestinal epithelial cells cannot directly absorb cholesterol ester, the role of CEase in absorption of dietary cholesterol is essential (Howles *et al.*, 1996). As shown in Fig. 2, all the four plant extracts showed significant inhibitory effect on CEase ranging from 19.67% to 51.60%. Tea extract showed the lowest inhibition (19.67%) followed by Hawthorn (23.14%), wolfberry (31.15%). Dendrobium extracts (51.60%) showed the strongest inhibition effect. Kumar *et al.* (2011) evaluated the *in vitro* cholesterol esterase enzyme inhibition of methanol extract of the leaves of *Camellia sinensis* (L.) and found that extracts has the ability to inhibit the enzyme with IC_{50} (82.46±0.74 µg/mL) and Zhao *et al.* (2018) reported that the cholesterol inhibition activity of black wolfberry seed oil with IC_{50} was 2.63 mg/mL. The shift in our results is perhaps due to the method of extraction and the different plant material used.

Fig. 2. Inhibition of different plants material on Cholesterol esterase activity (Cease), the CEase inhibition values measured in the four extracts were significantly different from each other ($p<0.05$).



Cholesterol micellization activity: The principal steps in the absorption of dietary cholesterol are emulsification, hydrolysis of the ester bond by a pancreatic esterase, micellar solubilization, and absorption in the proximal jejunum (Hui and Howles, 2005). In addition, Kirana *et al.* (2005) reported that the reduction of cholesterol absorption by reducing the solubility of cholesterol micellization in the intestinal lumen is a new target site of intervention for the treatment of hyperlipidemia and obesity. As shown in Fig. 3, our findings showed a significant difference on cholesterol micellization inhibitory activity ranging between 19%-42%.

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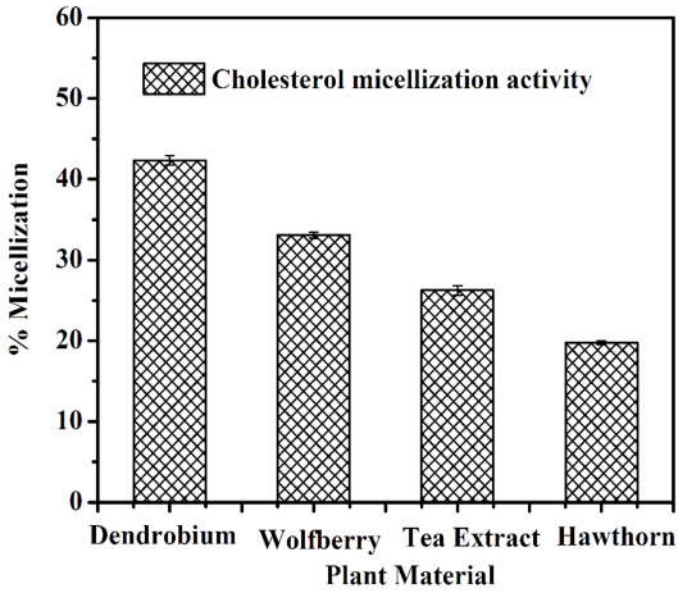
Among the four plant extracts, Dendrobium showed strong cholesterol micellization inhibition (42.33%) followed by Wolfberry (33.08%) and Tea extract (26.33%) with moderate inhibitory activity. Hawthorn extract (19.73%) showed the lowest cholesterol micellization inhibitory activity (Fig. 3).

Fig 3. Effect of different plant extracts on the cholesterol micelles solubility, all solubility values of cholesterol micellization measured in the four plant extracts were significantly different from each other ($p < 0.05$).



Conclusion

In conclusion, the results of our study showed that edible plant extracts have the ability to delay digestion and fat absorption through gastrointestinal mechanisms such as pancreatic lipase inhibition and cholesterol esterase inhibition as well as inhibition of cholesterol micellization.

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