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

Kaba Mamadi Aissata1,2,3,*, Lv Wenping1,2, Toure Samba Lamine1, Cliff J. Barra1, Sidibe Sidikiba3, Bertrand Muhoza1, Bibole Lubamba Maguy1 and Ma chaoyang1

1State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi 214122, PR, China; 2National Engineering Research Center for Functional Food, Jiangnan University, Wuxi 214122, PR, China; 3Faculty of Health Sciences and Techniques, Gamal Abdel Nasser University of Conakry, Guinea Faculty of Health Sciences and Techniques, BP: 1117

fstsugancrg@gmail.com*; +86 13861781146; Fax: + 86 5108591799

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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 macrovascular 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 (Brod 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...
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 officinale. 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 $2.40 \times 10^5$ 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 polyphenol 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 immunomodulating 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 ($8 \times 300$ mm i.d., YMC Co. Ltd, Kyoto, Japan) or Shodex OH pak SB-804 column ($8 \times 300$ mm 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 polyphenols:** The amount of total phenolics contents in extracts of Dendrobium, Wolfberry, Hawthorn and Tea extract was determined according to the
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≤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 kDa. 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 2.40×10^3Da, 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).

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Molecular Weight Distribution (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>40-65 KDa</td>
</tr>
<tr>
<td>Dendrobium</td>
<td>53.02</td>
</tr>
<tr>
<td>wolfberry</td>
<td>50.88</td>
</tr>
<tr>
<td>Hawthorn</td>
<td>16.21</td>
</tr>
<tr>
<td>Tea</td>
<td>16.21</td>
</tr>
<tr>
<td></td>
<td>3-4 KDa</td>
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<tr>
<td></td>
<td>62.78</td>
</tr>
<tr>
<td></td>
<td>28.41</td>
</tr>
<tr>
<td></td>
<td>45.44</td>
</tr>
<tr>
<td></td>
<td>1-2 KDa</td>
</tr>
<tr>
<td></td>
<td>20.21</td>
</tr>
<tr>
<td></td>
<td>20.71</td>
</tr>
<tr>
<td></td>
<td>38.35</td>
</tr>
<tr>
<td></td>
<td>0.1-0.5 KDa</td>
</tr>
<tr>
<td></td>
<td>20.21</td>
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<tr>
<td></td>
<td>20.71</td>
</tr>
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<td></td>
<td>38.35</td>
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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 (Ducuzseau 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>
<thead>
<tr>
<th>Plant extract</th>
<th>Mean ±SD (mg/g)</th>
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<tbody>
<tr>
<td>Hawthorn</td>
<td>11.3±0.62</td>
</tr>
<tr>
<td>Tea</td>
<td>6.17±0.51</td>
</tr>
<tr>
<td>Wolfberry</td>
<td>20.9±0.98</td>
</tr>
<tr>
<td>Dendrobium</td>
<td>25.2±1.22</td>
</tr>
</tbody>
</table>

**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.

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</tr>
<tr>
<td>Wolfberry</td>
<td>1.26±0.20</td>
</tr>
<tr>
<td>Dendrobium</td>
<td>1.13±0.20</td>
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**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≤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.

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**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%).

*Corresponding author
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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 IC50 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.

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 IC50 (82.46±0.74 μg/mL) and Zhao et al. (2018) reported that the cholesterol inhibition activity of black wolfberry seed oil with IC50 was 2.63 mg/mL. The shift in our results is perhaps due to the method of extraction and the different plant material used.

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%.

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 esterase inhibition: The Cholesterol esterase enzyme (CEase) hydrolyzes cholesterol esters to cholesterol and free fatty acid prior to their absorption.
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 micelles 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.

References

*Corresponding author
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