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A PHENOLIC-RICH HERBAL TEA FROM *Stathmostelma sp.* (STATROLTEA) INHIBITS *IN VITRO* PANCREATIC LIPASE ACTIVITY

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Abstract- The inhibitory effects of Statroltea, a potential functional food beverage produced from the leaves of *Stathmostelma sp.,* on pancreatic lipase activity were investigated using the pH-Stat technique. Results showed that Statroltea exerted a competitive inhibition on porcine pancreatic lipase activity in a dose dependant manner. A half enzyme activity was inhibited with a concentration of Statroltea (IC_{50}) of 1.04 mg/mL and the maximal inhibition (IC_{100}) was observed at 2.0 mg/mL. Statroltea at this latter concentration (2.0 mg/mL) exerted total inhibition against enzymatic hydrolysis of corn oil, sunflower oil, soybean oil, palm kernel oil meanwhile the inhibition of the hydrolysis of palm oil, olive oil and peanut oil was not complete. However, Statroltea had similar IC_{50} than catechin thus suggesting that natural Statroltea can be recommended as a lipase activity inhibitor for treating diet-induced obesity in humans rather than this synthetic compound. Results from fractionation showed that phenolic compounds found in Statroltea were responsible of the pancreatic lipase inhibition observed.

Keywords- Pancreatic lipase, inhibition, phenolic compounds, *Stathmostelma sp*., Statroltea

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Introduction

Excess energy intake and reduced energy expenditure result in abnormal excessive growth of adipose tissue, which can lead to the development of obesity [1,2]. Obesity is strongly associated with metabolic syndrome characterized by the presence of insulin resistance, hypertension, and hyperlipidemia [3]. One of the most important strategies in the treatment of obesity includes the development of inhibitors of nutrient digestion and absorption, in order to reduce the energy intake through gastrointestinal mechanisms, without altering any central mechanism [4,5]. Pancreatic lipase, the main lipolytic enzyme secreted by the pancreas, is greatly involved in the digestion of triglycerides. In fact, pancreatic lipase is responsible for the hydrolysis of 50-70% of total dietary fats [6] and pancreatic lipase inhibition is one of the most widely studied mechanisms for the determination of the potential efficacy of natural products as antiobesity agents [7]. Therefore, pancreatic lipase inhibitors are considered to be valuable therapeutic agents for treating diet-induced obesity in humans. Among the latter, the only clinically approved pharmacologic agent as pancreatic lipase inhibitor is Orlistat (Xenical®) whose active principle is tetrahydrolipstatin [8]. It is currently utilized as an effective aid in the management of human obesity [9]. Orlistat, however, possesses several unpleasant adverse reactions that have been shown to compromise the patients' compliance. These adverse reactions triggered a wealth of studies that searched for natural inhibitors of the pancreatic lipase with comparable efficacy to that of Orlistat, but void of its side effects.

Naturally-occuring polyphenols have been implicated in the inactivation of pancreatic lipase [10]. Because Phenolic compounds are widespread constituents of tea and herbal sources [7], many phenolic-rich herbal teas or infusions have been produced and drunk (green tea, mate tea, etc.) for the purpose of weight reduction through inhibition of pancreatic lipase activity. Nakai, et al [11] reported inhibitory effects of Oolong Tea Polyphenols on Pancreatic Lipase *in vitro*. Green tea extract and tea catechins have been reported to inhibit pancreatic lipase. *In vitro* experiments under gastric and duodenal conditions showed that fat digestion was significantly inhibited by inclusion of 60 mg Epigallocatechin gallate/g substrate due to some modifications in lipid emulsification in gastric or duodenal media [12].

A phenolic-rich beverage from the leaves of *Stathmostelma sp*. is consumed by some indigenous in northern regions of Cameroon for the purpose of weight reduction but the mechanism of action is still undetermined. This work intends to investigate the potentiality of the infusion of roasted leaves of *Stathmostelma sp*. called Statroltea as inhibitor of pancreatic lipase activity.

Materials and Methods

Preparation of Statroltea Solution

Fresh leaves of *Stathmostelma sp.* were harvested in Ngaoundere, Cameroon and identified at the Cameroonian national herbarium (Voucher N° 59014). Leaves were roasted at 144°C for 20 min to give *Stathmostelma sp.* roasted leaves tea (Statroltea) whose total phenolic content (TPC) was of 20.06 gallic acid equivalent (GAE) % dw determined as described by Dewantoo, et al [13]. After cooling at room temperature, roasted leaves were ground and bagged. Statroltea bags containing 3g of roasted leaves were brewed for 23 min in 300 mL of pre-heated water at 60°C. The TPC in the infusion was found to be 93.67 µg GAE/mL. The Statroltea infusion was dried at 40°C to obtain a dried residue that was then dissolved in pH 8 Tris-HCl buffer (1 mM Tris-HCl buffer, 150 mM NaCl and 5 mM $CaCl_2$) to obtain Statroltea solution.

In Vitro Enzyme Activity Assays

Lipase activity was assayed by measuring the fatty acids released from mechanically stirred emulsions of soybean oil in presence of pancreatic lipase, using 0.02N NaOH with a pH-stat (Metrohm 718 STAT Titrino, Switzerland), adjusted at an end point value of pH 8. Kinetic assays were performed in a thermostated (37°C) vessel containing 30 mL of oil emulsion prepared by mixing 2 mL of soybean oil and 58 mL of a pH 8 emulsifying buffer (1 mM Tris-HCl buffer, 150 mM NaCl, 5 mM $CaCl_2$ and 4 mM Sodium taurodeoxycholate). The reaction was started by introducing 750 µL of pancreatic lipase solution (8 mg/mL) obtained by dissolving the enzyme in pH 8 Tris-HCl buffer (1 mMTris-HCl buffer, 150 mM NaCl and 5 mM $CaCl_2$).

The inhibition was conducted by adding 100µL of a Statroltea solution to the oil emulsion before starting the reaction with the enzyme solution. Porcine pancreatic lipase was selected due to its high homology to the human enzyme (85% homology) and similar enzyme kinetics and behavior [14].

Comparison of Statroltea with Chemical Lipase Inhibitors

Inhibitory solutions were prepared with various inhibitors (Statroltea, catechin, saponin, THL) dissolved in Tris-HCl buffer to give a final concentration of 0.5 mg/mL. 100 µL of the inhibitory solution were melted into 30 ml of oil emulsion and the pH was adjusted to 8. The lipase activity was assayed by measuring the fatty acids released from mechanically stirred oil emulsion after injection of 750 µL of a pancreatic lipase solution. Kinetic assays were performed in a thermostated (37 °C) vessel, using 0.02N NaOH with a pH-stat, adjusted at an end point value of pH 8. The IC_{50} was determined as the concentration of the inhibitor leading to 50% of inhibition.

Influence of Fatty Acids Composition of Oils on Statroltea Inhibitory Activity

Vegetable Oils used were soybean oil, corn oil, sunflower oil, olive oil (Dumortier Huiles et Sauces, Rotterdam), peanut oil (Sitron, Garoua, Cameroon), palm oil and palm kernel oil. These oils were purchased in the local market. The different oil emulsions were obtained by mixing 1 mL of oil to 29 mL of the emulsifying buffer pH 8, and then inhibition was conducted by adding 320 µL of a Statroltea solution (0.5 mg/mL). Lipase activity was assayed by measuring the fatty acids released from mechanically stirred oil emulsion after injection of 750 µL of a pancreatic lipase solution. Kinetic assay were performed in a thermostated (37°C) vessel, using 0.02N NaOH with a pH-stat, adjusted at an end point value of pH 8. The percentage of inhibition was evaluated.

Effect of Phenolic Compounds of Statroltea on *In Vitro* Pancreatic Lipase Activity

Statroltea (25g) was stirred twice for 1 hour in 250 mL of water. The extract was collected and the residue discarded. The aqueous phase was partitioned in 250 mL of chloroform, and then the two phases were separated, dried at 40 °C and dissolved in Tris-HCl buffer to be tested against *in vitro* pancreatic lipase activity. Furthermore, the water soluble fraction was fractionated on a Sephadex LH 20 column (30 x 2.5cm) chromatography using the method described by Takako and Takako [15]. Briefly, Sephadex LH20 was swollen in water, poured into a glass column and equilibrated with water. The fractionation was conducted by successive application of 200 mL of water and ethanol (80%). These solvents were chosen for their safety for human consumption and the best ethanol concentration (80%) was selected based on the amount of total phenolic compounds extracted in preliminary tests. Then, the column was washed twice with acetone (50%). The resulting fractions were used first for pancreatic lipase inhibition assay and secondly to identify their TPC by the Folin- Ciocalteau method as described by Dewantoo, et al [13]. After plotting these two measurements, the coefficient of determination expressing their relationship was obtained through the tendency curve.

Results

Effect of Statroltea on the Inhibition of Pancreatic Lipase

The inhibitory action of Statroltea against porcine pancreatic lipase was determined using different concentrations of Statroltea (0.5-2.0 mg/mL). As shown in [Fig-1], Statroltea inhibited the enzyme activity in a dose-dependent manner with a concentration for half maximal activity (IC_{50}) of 1.04 mg/mL. The inhibition was complete and the maximum of inhibition (100%) was obtained at 2.0 mg/mL.

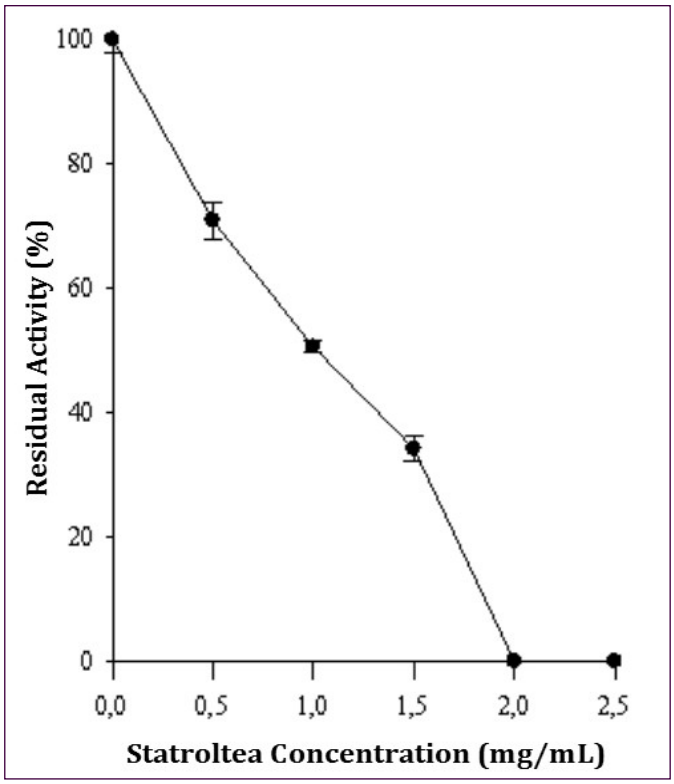

Fig. 1- Effect of Statroltea on the activity of pancreatic lipase

Compared Potency of Statroltea and Some Chemical Lipase Inhibitors

[Fig-2] shows the inhibitory effects of Statroltea and some pure compounds on pancreatic lipase. Tetrahydrolipstatin (THL), a lipase inhibitor used as the positive control, strongly inhibited pancreatic lipase activity with an IC_{50} of 0.11 mg/mL. In fact, the tested compounds inhibited the activity of porcine pancreatic lipase with IC_{50} of: 0.11 mg/mL for THL, 0.72 mg/mL for saponin, 0.92 mg/mL for catechin and 1.04 mg/mL for Statroltea.

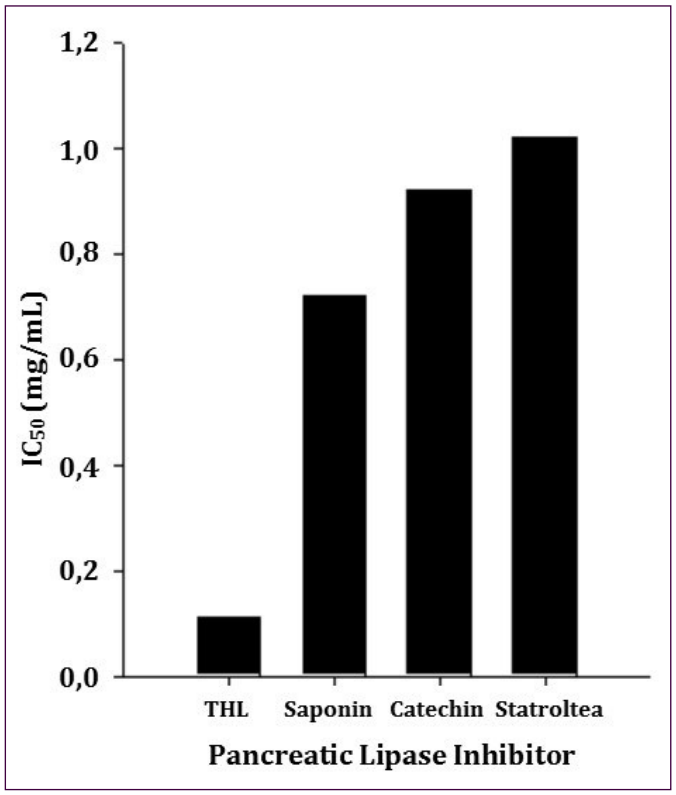

Fig. 2- Compared anti-lipase activity of Statroltea and some synthetic compounds

Effect of Fatty Acids Composition of Some Oils on Statroltea Inhibitory Activity

Pancreatic lipase is a key enzyme in dietary triacylglycerol absorption, hydrolyzing triacylglycerols to 2-mono-acylglycerol and fatty acids. The inhibitory effect of Statroltea on pancreatic lipase in respect to the fatty acid composition of the substrate was investigated. [Table-1] reveals that Statroltea at a concentration of 2.0 mg/mL was found to be more potent in the inhibition of the hydrolysis of corn oil, sunflower oil, soybean oil, palm kernel oil emulsions (100% of inhibition). However, the inhibition of the hydrolysis of palm oil, olive oil and peanut oil was not complete with this same concentration of Statroltea.

Table 1- Rate of inhibition of hydrolysis of some oils

Types of oils	SFA (%)	MUFA (%)	PUFA (%)	Inhibition (%)
Palm kernel oil	81.9	16.4	3.1	100
Corn oil	14.8	28.1	57.1	100
Soybean oil	15.7	24.2	59.8	100
Sunflower oil	12.8	22.4	66	100
Palm oil	50.4	39.4	10.5	77.78
Peanut oil	18.3	49.6	30.8	66.67
Olive oil	15.3	73.8	10	53.69

The activity of the enzyme in the presence of Statroltea (2.0 mg/mL) varied with the type of substrates and thus with the type of fatty acids involved in the composition of these substrates. There is a strong negative correlation (r = - 0.97, plot not shown) between the enzyme activity in presence of Statroltea and the proportion of monounsaturated fatty acids contained in the substrate.

Fractionation

[Fig-3] exhibits the bioactivity- guided fractionation of Statroltea. It can be seen that the chloroform soluble fraction had no activity against porcine pancreatic lipase activity whereas the water soluble fraction was active. The fractionation through Sephadex LH20 revealed that the fraction obtained after elution by ethanol 80% was more potent in the inhibition of pancreatic lipase activity and was found to possess the highest TPC (74.65 ± 2.23 % dw). The coefficient of determination (plot not shown) that reveals the association between the TPC of the fractions and their IC_{50} was found to be 98.41% thus proving that a strong relationship could be established between the potency of pancreatic lipase inhibition and the phenolic constituents in Statroltea.

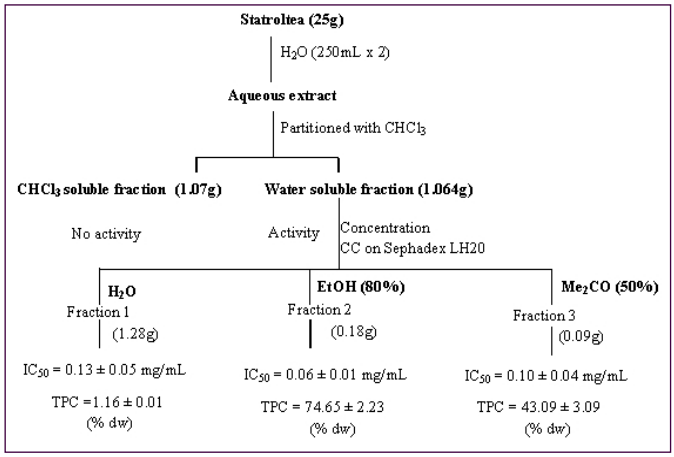

Fig. 3- Bioactivity- guided fractionation of Statroltea

Discussion

These results indicated that Statroltea with an IC_{50} of 1.04 mg/mL and an IC_{100} of 2.0 mg/mL was more effective in inhibiting pancreatic lipase than some herbal teas, as Martins, et al [6] showed that mate tea obtained from leaves of *Ilex paraguarinensis* inhibited pancreatic lipase with an IC_{50} of 1.5 mg/mL. Han, et al [10] reported that a concentration of 2.0 mg/mL of a saponin fraction produced individually from oolong, green and black tea respectively produced inhibition ratios of 100, 75 and 55%. Differences in the effectiveness of these teas were probably due to their chemical compositions especially the phenolic content and phenolic composition, as many studies have focused on polyphenols from teas and herbal sources and their effects against pancreatic lipase activity [2,7].

Chemical compounds (THL, saponin and catechin) were found to inhibit pancreatic lipase activity. Similar results were reported by Ransac, et al [16] who found that the surface concentrations of THL, which reduces lipase activity to 50%, are 0.013% with porcine pancreatic lipase. Pure compounds like THL and saponin are more potent than Statroltea which is a crude extract containing various constituents. Kim and Kang [18] reported that crude extracts include, not only active substances, but also non-active components, thus explaining why the inhibitory potencies of crude extracts from plants and other natural sources are reduced compared to pure

chemical molecules. In the other hand, Ikarashi, et al [17] reported that Acacia Polyphenol whose major constituents are robinetinidol and fisetinidol with structures similar to catechin was found to inhibit pancreatic lipase activity with an IC_{50} of 0.95 mg/mL. This is concomitant with our results which showed that the IC_{50} of catechin was found to be 0.92 mg/mL. However, Statroltea had comparable IC_{50} to catechin, thus suggesting phenolic compounds in Statroltea may have similar structures than the phenolic catechin and that natural Statroltea can be recommended as a lipase activity inhibitor rather than this synthetic compound.

The enzyme activity in presence of Statroltea was found to be negatively strongly correlated with the proportion of monounsaturated fatty acids contained in the substrate. This finding was consistent with the observations of Ikeda, et al [19] which showed that the inhibition of fat absorption by catechin, a phenolic compound found in green tea was both moderate and dependent on the types of fat incorporated into lipid emulsions. Usually, pancreatic lipases are 1,3 -positional-specific which means that reactions involving triacylglycerol hydrolyses hydrolysis are confined to the sn-1 and sn-3 positions and the sn-2 acyl groups remain unaltered. In most vegetable oils, the monounsatured fatty acid oleic acid is predominantly at the sn-2 position in the triacylglycerol species [20]. This stereospecificity of porcine pancreatic lipase may explain why the hydrolysis of an oil rich in monounsaturated fatty acids will be less complete than of oils rich in saturated fatty acids or polyunsaturated fatty acids.

A strong relationship could be established between the potency of pancreatic lipase inhibition and the phenolic constituents in Statroltea. This finding was consistent with McDougall, et al [21] who reported that berry polyphenols inhibit pancreatic lipase activity *in vitro*. In the same line, Martins, et al [6] stated that polyphenols in mate tea were responsible for the pancreatic lipase inhibition.

Conclusion

A number of studies have revealed various health benefits of plant polyphenols and their potentialities in foods, beverages and natural medicine. In this light, polyphenols have some potential efficacy for preventing obesity through pancreatic lipase inhibitory effect. Results from our study showed that an herbal tea from *Stathmostelma sp.* (Statroltea) efficiently inhibited *in vitro* porcine pancreatic lipase activity with an IC_{50} of 1.04 mg/mL. Fractionation of Statroltea showed that the beverage contained phenolic compounds involved in the inhibition of pancreatic lipase activity. Up to date, effects of Statroltea have not been reported on lipase activity and our study demonstrated that phenolic-rich Statroltea from *Stathmostelma sp.* leaves had a potential effect as anti-obesity herbal tea. Thus, it is worthwhile to further investigate this herbal tea for its potential pharmacological effect as antiobesity agent and an attempt should be made to characterize phenolic compounds to be used as safe therapeutic agents in future.

Conflict of Interest : None declared.

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