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Research Article

IN VITRO REDUCING POWER PROPERTY AND PANCREATIC LIPASE INHIBITORY ACTIVITY OF LABORATORY MADE *DASAMOOLARISHTAM*

Narayanasamy A and Sudheer Mohammed M.M*

Pharmacognosy & Phytochemistry Laboratory, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore-18, Tamil Nadu, India

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ABSTRACT

Ayurveda comprises of various type of formulations with respective therapeutic values. The quality assurance of the final product is an important issue leading to the reduction of the popularity when compared to the western medicine. Single or combination of herbal formulations have been used longtime by humans. And their measurement of healing properties with the application of modern methods are inevitable in present days. *Dasamoolarishtam* is one of the most useful oral formulation prescribed in Ayurveda for various ailments and it possess variety of phytochemicals. The present study was aimed to find out the anti-oxidant and anti-obesity activities of *Dasamoolarishtam* sample prepared by direct ethanol solvent (DET) extraction with the comparison of marketed *Dasamoolarishtam* (MKT). Both the samples showed good results in reducing power ability and pancreatic lipase inhibition activities. However, the DET samples showed higher activity than MKT in both the studies. It was observed that absorbance value 0.788 ±0.100 in DET and 0.733 ±0.106 in MKT at 700nm in reducing power assay. IC_{50} value of DET was 31.09 ±3.14 µg/ml in pancreatic lipase effect which was observed as 38.58 ±2.80 µg/ml in MKT. The results were compared with respective reference compounds. The findings of the present study clearly states that the DET extract has comparatively promising results than MKT. Therefore, the present study recommended that the DET preparation could be a new source for the treatment of oxidative damage and obesity problems when it will be prepared as a medicine after the clinical evaluation.

INTRODUCTION

The use of herbal drugs for the treatment of various ailments is an orthodox practice[1] still followed globally. Traditional systems of medicine have a very long, safe and continuous usage and efficacy which are officially recognized as alternative systems of health such as Ayurveda, Unani, Siddha, Homeopathy and Naturopathy[2]. Generally Ayurvedic formulations are a mixture of multi components including plants, animal products, minerals and metals [3]. In Ayurveda, many medicinal plantsare used in herbal preparations (polyherbal formulations) and proposed for their interesting multilevel activities[4]. Ayurvedic polyherbal formulations are based on the fact that the therapeutic efficiency of the herbal preparations enhanced by the synergistic efficacy of the member plants [3]. Among various Ayurvedic drugs, the *choornams*, *kashayams*, *lehyams*, *arishtas*, *asavas*, *guikas*/*pills*, *thylam* or oil extractions are commonly used [5]. A few Ayurvedic drugs have only been scientifically investigated for their chemical profiling and therapeutic values, but most of them still need to be explored.

The curative effect of herbal drugs dependent on the active phytoconstituents available in the herbs used. The most bioactive phytoconstituents are secondary in origin specially meant for plant defence. The secondary metabolites are bioactive constituents which are available in minimum quantity in plant parts and their site of synthesis and place of storage varies. In human system, overloaded free radicals damage the body cells and cause several diseases like cardiovascular disorders, lung damage, inflammation etc. Antioxidant molecules are capable of reducing or preventing the harmful oxidation process of other molecules. Thus, there is anurgent need for morenatural antioxidants supplied to human body because they can protect the human body from the diseases caused by free radicals[6, 7]. Electron donor compounds can reduce the oxidized intermediates of lipid peroxidation processes which indicate that they have potent reducing power. So these compounds can act as primary and secondary antioxidants[8].

Pancreatic lipase is a key enzyme for the absorption dietary triglycerides[9], it performs 50–70% of hydrolysis of total dietary fats [10]. Inhibition of pancreatic lipase enzyme is the key mechanism for the reduction of fat absorption resulting in the

Corresponding author:* **Sudheer Mohammed M.M
Pharmacognosy & Phytochemistry Laboratory, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore-18, Tamil Nadu, India

regulation of obesity[10]. Plant based sources are an exciting opportunity for the discovery of newer antioxidants[11, 12] and anti-obesity[13, 14] agents.

Dasamoolarishtam is an Ayurvedic polyherbal *arishta* formulation traditionally prepared by the mixture of 67 plants with the addition of water, sugar, ghee, honey and kasthuri gland (secretion of musk deer)[15]. This formulation is traditionally used to manage many ailments including post-natal care, stomach problems, blood and circulatory system problems, respiratory diseases, etc., and it also used as a general health tonic[16]. This formulation already reported to possess many biological properties[16]. As per the available literatures, this formulation is not yet to investigated for its *invitro* anti-obesity activity.

Medicinal plant trade is an important alternative income generating source for many developing countries. Thus there is an urgent need to scientific validation of traditional (Ayurvedic) medicines for the accepting international regulatory guideline to get drug approval status[17]. Classical Ayurvedic drugs need contemporary modifications, because of the change of human life style and the evolution of microbial pathogens [18]. Therefore the standardization and modifications in Ayurvedic drugs are preferable topic of research in recent days. Therefore, the present research was aimed to discover the free radical reducing power ability and anti-obesity activity of marketed *Dasamoolarishtam* (fermented decoction) and laboratory made ethanol based plant only (modified) *Dasamoolarishtam* preparations.

MATERIALS AND METHODS

Collection and identification of plant materials

List of plantspecimens and sample parts used for the *Dasamoolarishtam* preparation was collectedas per the standard publications[15, 19, 20, 21]. Fresh plant specimens were collected from various parts of Coimbatore and Nilgiris districts of Tamil Nadu, and some of the dry specimens were purchased from herbal dealersin Coimbatore city. All the specimens were identified in our laboratory by using various markers[22, 23, 24, 25, 26]. The marketed *Dasamoolarishtam* preparation was procured from a local herbal shop in Coimbatore city.

Preparation of the extract of Dasamoolarishtam ingredients

The collected plant materials were washed with running tap water until the removal of all the surface debris and were dried in shade condition. The dried specimens were coarsely powdered and stored in air tight sterile bottles for extraction. Required quantity of plant powders[27] were taken and mixed together, and extracted with sufficient volume of ethanol (99%) solvent by using Soxhlet apparatus for 72 h. This extraction (DET) was performed without water, honey, sugar, ghee and kasturi, which are non-plant ingredients of the *Dasamoolarishtam*. In contrast, commercially available marketed preparation (MKT) contain both the plant and non-plant ingredients.

Determination of Reducing power ability

The $Fe3^{+}$ reducing power of DET and MKT preparations were determined according to the method suggested by Oyaizu [28]. Different concentration of test samples (100, 200, 300, 400, 500 µg/ml) were mixed with 5.0 ml of 0.2M phosphate buffer (pH-6.6) and 5.0 ml of 1% potassium ferricyanide, then the mixtures were incubated at 50°C for 20 min. The reaction was terminated by adding 5.0 ml of 10% 2,4,6-trichloroanisole (TCA) (w/v), and the mixture was centrifuged at 1000 rpm for 10 min. The upper layer of the supernatant (5.0 ml) was mixed with 5.0 ml of distilled water and 1.0 ml of 0.1% (w/v) $FeCl_3$ solution. The absorbance was measured at 700nm in a spectrophotometer. Rutin served as reference compound. Increased absorbance indicates increased reductive capability.

Determination of Pancreatic lipase inhibitory activity

DET and MKT samples were examined for anti-obesity activity by using pancreatic lipase inhibition method established by Kim *et al*. [29] and Roh and Jung[30] with slight modifications. Fresh enzyme buffer was prepared by adding 6µl of porcine pancreatic lipase solution (1mg/ml) in buffer containing 10mM 3-morpholinepropane-1-sulphonic acid and 1mM EDTA (pH-6.8), with 164 µl of Tris buffer (100mM Tris-HC1 and 5 mM CaCl2, pH-7.0). Then, 20µl of various concentration (100, 50, 25, 12.5, 6.25, 3.125, 1.565 µg/ml) of either the *Dasamoolarishtam* samples or orlistat were mixed with 170µl of enzyme buffer on a 96 well-quartz microplate and then the plate was incubated at 37°C for 15 min. After that, 10 µl of substrate solution [10mM p-NPB (p-nitrophenyl butyrate in dimethyl for mamide)] was added and incubated for 15 min at 37°C. The lipase activity was determined by measuring the hydrolysis of p-NPB to p-nitrophenol. The absorbance was measured at 405nm using a UV/Visible micro-plate spectrophotometer. The inhibition of lipase activity by the samples was calculated according to the following formula:

$$\text{Lipase inhibition (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}}$$

The IC_{50} value was calculated as the concentration of the sample extract to inhibit 50 % of pancreatic lipase inhibitory activity under assay condition.

Statistical analysis

Triplicate values were done for every experiments, and the results were expressed as mean ± standard deviation. The data were statistically analysed using SPSS version 20.0, employing one-way ANOVA followed by Duncan's Multiple Range test for *in vitro* studies. Mean values were measured statistically significant at $p<0.05$.

RESULTS

Antioxidant activity using the Reducing power ability method

Table 1 showed the result of reducing power assay of DET and MKT samples. Among them, the maximum reducing power (absorbance value 0.788 ±0.100 at 700nm) was observed in DET at high concentration (500µg/ml). The MKT sample showed its maximum absorbance as 0.733 ±0.106 at high concentration. Much higher reducing capability was observed in rutin (1.118 ±0.007 in 500µg/ml). The results are concentration dependent manner, if the concentration is increased the activity also increased.

Table 1 Reducing power ability of different concentrations of test samples

Samples	Absorbance at 700 nm				
	100 (µg/ml)	200 (µg/ml)	300 (µg/ml)	400 (µg/ml)	500 (µg/ml)
DET	0.424 ±0.004	0.553 ±0.004 [d]	0.573 ±0.004 [d]	0.656 ±0.103 [c]	0.788 ±0.100 [b]
MKT	0.352 ±0.003	0.408 ±0.005	0.523 ±0.008 [d]	0.615 ±0.105 [c]	0.733 ±0.106 [b]
Rutin	0.675 ±0.103 [c]	0.773 ±0.102 [b]	0.844 ±0.103 [b]	1.012 ±0.103 [a]	1.118 ±0.007[a]

DET- Direct Ethanol Extract, **MKT**- Marketed Sample.

Values are mean of triplicate determination (n=3) ± standard deviation.

Statistically significant at $p<0.05$ where $^{a}>^{b}>^{c}>^{d}$ in each column.

Anti-obesity activity using the Pancreatic lipase inhibitory effect

Various concentrations of DET and MKT preparations were tested for anti-obesity activity. The pancreatic lipase inhibitory effect of the samples were indicated by percentage of inhibition and IC_{50} values (table 2 and figure 1). The samples inhibited the activity in a concentration dependent manner. The DET sample had the highest activity as 79.01 ±2.34% at 100 µg/ml concentration followed by MKT (74.46 ±6.91%). The standard orlistat showed maximum inhibition (85.70 ±3.70) percent at 100 µg/ml. However, the *Dasamoolarishtam* preparations exhibited significant anti-obesity activity. The IC_{50} value was found to be at 31.09 ±3.14, 38.58 ±2.80and 20.53 ±1.75 µg/ml in DET, MKT and orlistat samples respectively.

Polyphenols especially flavonoids and tannins were reported to possess great therapeutic values including anti-inflammatory, antimicrobial, anticancer, antihypertensive, anti-allergic, antioxidant and anti-obesity activities [31, 32, 33, 34]. Hence, the present study was designed to reveal the anti-obesity activity and reducing power ability of DET and MKT samples.

Reducing power of the crude drug may be due to the ability of hydrogen donation that stabilize the molecules by acceptance of hydrogen ions from the crude drugs [35]. Antioxidant property of a compound can be indicated by reducing power ability of the compound evaluated[36]. The reducing power of DET and MKT increases with the increase in amount of sample concentrations.

Table 2 Effect of DET and MKT sampleson Pancreatic lipase inhibition

Samples	% of Inhibition						
	1.565 (µg/ml)	3.125 (µg/ml)	6.25 (µg/ml)	12.5 (µg/ml)	25 (µg/ml)	50 (µg/ml)	100 (µg/ml)
DET	18.09 ±0.87	29.18 ±5.19	38.12 ±3.46	47.11 ±1.12 [e]	59.23 ±4.57 [d]	68.93 ±2.71 [c]	79.01 ±2.34 [b]
MKT	17.92 ±5.93	26.39 ±5.06	33.67 ±0.12	41.6 ±4.94 [e]	55.77 ±7.78 [d]	63.28 ±3.95 [c]	74.46 ±6.91 [b]
Standard (Orlistat)	23.51 ±1.85	33.90 ±1.23	42.46 ±1.41 [e]	53.60 ±4.94 [d]	64.93 ±2.71 [c]	76.46±0.14 [b]	85.70 ±3.70 [a]

DET- Direct Ethanol Extract, **MKT**- Marketed Sample.

Values are mean of triplicate determination (n=3) ± standard deviation.

Statistically significant at $p<0.05$ where $^{a}>^{b}>^{c}>^{d}>^{e}$ in each column.

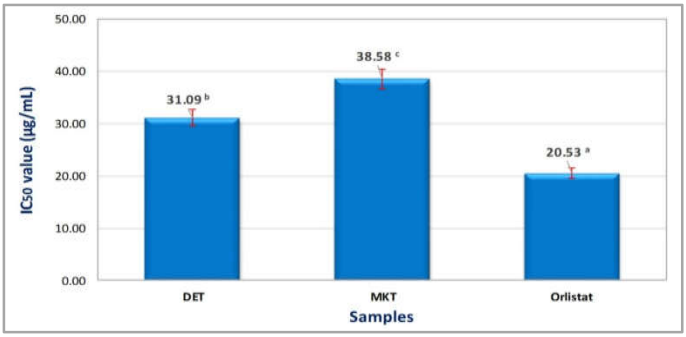

Figure 1 IC_{50} value of Pancreatic lipase inhibitory activity of DET and MKT samples

DET- Direct Ethanol Extract, **MKT**- Marketed Sample.
Values are mean of triplicate determination (n=3) ± standard deviation.
Statistically significant at $p<0.05$ where $^{a}>^{b}>^{c}$ in each column.

DISCUSSION

In a previous study[16], the laboratory mode *Dasamoolarishtam* and marketed *Dasamoolarishtam* preparations showed the presence of various phytochemicals (including flavonoids and tannins), and high amount of phenolics, tannins and flavonoids content, and rich antioxidant (DPPH, ABTS, FRAP, Superoxide radical scavenging and Phosphomolybdenum) activity.

The reducing power showed good absorbance in high concentration at 700nm by DET. The DET sample already reported to contain high phenoliccontent[16] which is responsible for its higher reducing power activity.

Many research works were carried out in detail to know about the potential efficacy of natural products as anti-obesity agents [37]. Fatty acids and monoglycerides were derived from dietary fats bylipid hydrolysis process and forms mixed micelles with bile salts, cholesterol, and lysophosphatidic acid[29]. Then the mixed micelles absorbed into enterocytes where resynthesize of triglycerides happens. Finally, the triglycerides stored in adipocytes as the major source of energy [10].

Pancreatic lipase inhibition is one of the new approach for the treatment of obesity, tried to reduce energy intake through gastrointestinal mechanisms, without altering any vital mechanisms [29]. The percentage of inhibition and IC_{50} values of pancreatic lipase inhibitory effect of DET sample showed better anti-obesity activity over the MKT sample.

Tannins and flavonoids are most effective pancreatic lipase inhibitors[38, 39, 40], therefore high amount of these compounds found in DET extract[16]may be associated with its higher anti-obesity activity. To the best of our knowledge the present study could be a first report on the anti-obesity activity of *Dasamoolarishtam* formulation(s).

CONCLUSION

The present study was conducted to realize how the laboratory mode *Dasamoolarishtam* and marketed *Dasamoolarishtam* samples exert antioxidant and anti-obesity effects with the respect of reducing power and pancreatic lipase inhibition assays. From the results obtained in the present study, it was concluded that, the DET and MKT samples showed significant level of antioxidant and antiobesity activities. However, DET showed much stronger effects in both the activities, this DET sample could serve as a potent drug for the treatment of oxidative damage and hyper triglyceridemia (high fat assimilation). However, further studies are needed to verify the reduction of oxidative damage and anti-lipase inhibitory activity of both the preparations by using animal models.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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