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Evaluation of anti-obesity activity of *Gymnema sylvestre* leaves extract

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Abstract
Obesity is characterized as abnormal or excessive fat deposition in adipose tissue and is a chronic disorder of carbohydrate and fat metabolism and poses a risk to the health and well-being of humans. Natural herbal products for weight reduction may be effective in the treatment of obesity and associated disorders. Therefore, the present study was to investigate the phytochemical screening and anti-obesity activity of *Gymnema sylvestre* leaves extract. The phytochemical screening *Gymnema sylvestre* leaves showed that the presence of saponins, flavonoids, steroids, terpenoids, polyphenol, and coumarins whereas tannin and triterpenoids were absent in ethanol and aqueous extracts. Alkaloids and glycosides present only ethanol extract. Anthroquinones present only aqueous extract. Quantitative analysis revealed that the *Gymnema sylvestre* leaves has flavonoids, saponin, phenol and terpenoid. Significant amount of flavonoids (27.29 mg/gm), saponin (37.18 mg/gm) phenol (142.00 mg/gm) and terpenoid (31.00 mg/gm) were presented. The anti-obesity activity of *Gymnema sylvestre* proved by inhibition of lipase. Overall, it can be concluded from the present study that *Gymnema sylvestre* leaves contains rich source of phytochemicals and possess anti-obesity activity.

Keywords: *Gymnema sylvestre* leaves, phytochemical screening, anti-obesity activity

Introduction
Obesity is a chronic disease which has spread all over the world and threatens public global health. The phenomenon of obesity has drawn the attention of the scientific community, organizations and governments worldwide because it affects people's lives negatively and imposes excessive financial implications in every health system. In addition, obesity has been the major interest in health sciences and many research studies have focused not only on the prevalence and the risk factors of obesity but also on the significant consequences on the quality of patients' life. Furthermore, is associated with increased incidence of type 2 diabetes mellitus, hypertension, coronary heart disease, arthritis, sleep apnea, and certain forms of cancer (Ogden *et al.*, 2004) [1].
The prevalence of obesity has been increasing worldwide, which has a great impact on lifestyle-related disorders such as coronary heart disease, atherosclerosis, and diabetes. Excess visceral abdominal fat accumulation appears to be a key feature of abdominal obesity contributing to the development of the metabolic syndrome. Therefore, preventing abdominal fat accumulation is an ideal option for the treatment of obesity and related diseases. Plant and plant products play a wide range of biological properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. Keeping in view, the present study aimed to investigate the phytochemical analysis and anti-obesity activity of *Gymnema sylvestre* leaves extract.

Materials and Methods
Collection of plant materials
The leaves powder of *Gymnema sylvestre* were purchased in December 2018 from siddha medicinal shop, Thanjavur, Thanjavur district, Tamil Nadu, India.

Preparation of plant extract
1 gram of the powder of *Gymnema sylvestre* leaves were transferred in to different conical flask (250ml). The conical flask containing 50ml of different solution (ethanol and water). The conical flask containing *Gymnema sylvestre* leaves were shake it well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using whatman filter paper No.1 and filtrate used for further analysis.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973) [2-4].

Quantitative analysis of phytochemicals

Total phenols estimated by the method of Edeoga *et al*., (2005 [5]). Flavonoid determine by the method of Boham and Kocipai-Abyazan (1994) [6]. Saponin determine by the method of Obdoni and Ochuko (2001) [7]. Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956) [8].

***In vitro* anti-obesity activity**

Antiobesity activity carried out by Rashmi Shivanna *et al*., (2017) [9]. Different concentrations of sample was takes as 1, 2, 3 and 4 ml of each concentration of the juice was mixed with 8 ml of olive oil, 0.4 ml phosphate buffer and 1 ml of lipase and it were incubated for 60 min. The reaction was stopped by the addition of 1.5 ml of a mixture containing acetone and 95 % ethanol (1:1). The appearance of pink colour from yellow colour shows the liberated fatty acids, which was determined by titrating the solution against 0.02 M sodium hydroxide (standardized by 0.01 M oxalic acid) using phenolphthalein as an indicator and the percentage inhibition of lipase activity was calculated.

Results and Discussion

In the present study was carried out on the *Gymnema sylvestre* leaves revealed the presence of medicinally active constituents. The phytochemical characters of the *Gymnema sylvestre* leaves investigated and summarized in Table-1 and figure 7 and 8. The phytochemical screening *Gymnema sylvestre* leaves showed that the presence of saponins, flavonoids, steroids, terpenoids, polyphenol, and coumarins whereas tannin and triterpenoids were absent in ethanol and aqueous extracts. Alkaloids and glycosides present only ethanol extract. Anthroquinones present only aqueous extract.

Hassain *et al*. (2011) [10] screened phytochemical constituents from methanol leaf extract of *Bombax malabaricum*. Various organic 11 solvent extracts of *Pedalium murex* were subjected to preliminary phytochemical screenings by Thamizh mozhi *et al*. (2011) [11]. Selected 53 traditionally used medicinal plants from western region of India for their qualitative phytochemical screenings, total phenol and flavonoids contents Pascaline *et al*. (2011) [12].

Reena Ganesan *et al*., (2013) [13] aimed to carry out preliminary phytochemical of six different solvents extracts from leaf and leaf derived callus of *Sebastiania chamaelea*. The preliminary phytochemical analysis reflects the presence of phenolic compounds, carbohydrate, alkaloids, phytosterols, fats and oils, terpenoids. The result highlights among two extracts, leaf extract show negligible activity than callus extracts

Kumar *et al*., (2013) [14] investigated the preliminary phytochemical screening of the leaves of the plant *Lasia spinosa* (Lour) Thwaites. The phytochemical screening showed that the methanol and aqueous extracts contained alkaloid, the carbohydrates and the phenolic compounds were present in all of the solvent extract except petroleum ether extract. The chloroform, ethyl acetate and the aqueous extract contained glycosides whereas the saponins present in methanol and aqueous extract. The ethyl acetate extract contain only the flavonoids.

Table 1: Qualitative analysis of Phytochemicals in *Gymnema sylvestre* leaves

S. No	Phytochemicals	Extracts	
		Ethanol	Aqueous
1	Tannin	-	-
2	Saponin	+	+
3	Flavonoids	+	+
4	Steroids	+	+
5	Terpenoids	+	+
6	Triterpenoids	-	-
7	Alkaloids	-	+
8	Antroquinone	+	-
9	Polyphenol	+	+
10	Glycoside	-	+
11	Coumarins	+	+

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

Quantitative analysis

Quantitative analysis revealed that the *Gymnema sylvestre* leaves has flavonoids, saponin, phenol and terpenoid. Significant amount of flavonoids (27.29 mg/gm), saponin (37.18 mg/gm) phenol (142.00 mg/gm) and terpenoid (31.00 mg/gm) were presented (Table 2). The above phytoconstituents were tested as per the standard methods.

Table 2: Quantitative phytochemical analysis of *Gymnema sylvestre* leaves extract

S. No	Secondary Metabolites	Result (mg/gm)
1	Flavonoids	27.29±1.12
2	Saponin	37.18±0.59
4	Phenol	142.00±1.53
5	Terpenoid	31.00±1.28

Values are expressed as mean ± SD for triplicates

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials (Das *et al*., 2010) [15].

Leo Stanley *et al.* (2011) [16] reported that leaves of *C. pedata* showed the presence of alkaloids, carbohydrates, steroids, tannin, phenolic compounds, flavonoids and terpenoids. Dinesh kumar *et al.* (2011) [17] has been reported to terpenoids, flavonoids and tannin are present in *C. trifolia.* Rajmohanan *et al.* (2014) [18] investigated the preliminary phytochemical analysis of various extracts of leaves of *C. pedata* and showed the presence of carbohydrates, flavonoids, tannins and phenolic compounds and terpenes.

In vitro* anti-obesity activity of *Gymnema sylvestre

In the present scenario, obesity is the major public health problem with about 1.9 billion adults (18 years and older) worldwide are overweight and about 600 million of them are clinically obese (Centre, 2015) [19]. Obesity is characterized by increase in adipose cell size which is determined by amount of fat accumulated in the cytoplasm of adipocytes. This change in the metabolism in the adipocytes is regulated by various enzymes such as fatty acid synthase, lipoprotein lipase and adipocyte fatty acid-binding protein (Rosen *et al*., 2000) [20]. Thus, inhibition of digestion and absorption of dietary fat is a first key to treating obesity. This inhibition involve pancreatic lipase enzyme, the principle lipolytic

enzyme synthesized and secreted by the pancreas. Pancreatic lipase is important enzyme in dietary triacylglycerol absorption, hydrolyzing triacylglycerol to monoacylglycerol and fatty acid. Substrates for lipase enzyme are long-chain triacylglycerol, which are separated from the aqueous medium by the surface phase. Thus, lipase enzyme must be adsorbed on the substrate lipid surface and the nature of the surface of the substrate is an key role for lipase activity (Roh *et al*., 2012) [21].

Inhibition of pancreatic lipase is an attractive targeted approach for the discovery of potent anti-obesity agents for obesity treatment (Thomson *et al*., 1997; Tsujita *et al*., 1989) [22-23]. One of the screening strategies used in the discovery of anti-obesity drugs is to search for potent lipase inhibitors from plant extracts. Plants have been used as traditional natural medicines for healing many diseases. In particular, various oriental medicinal plants are reported to have biological activity (Shizhen and Xiwen, 2003; Rahul Birari *et al*., 2010) [24,25]. In this study, we screened *Gymnema sylvestre* leaves extract from natural sources as potential anti-obesity agents by monitoring their anti-lipase activity. As shown in Table 3, *Gymnema sylvestre* leaves extract inhibited lipase activity as concentration-dependently on the *in vitro* assay. *Gymnema sylvestre* leaves extract significantly inhibited lipase with low concentration was 30.20% while the higher concentration was 84.11% as compared about orlistat as standard drug. The highest concentration is nearest to the standard. Medicinal plants have played a vital role in inhibiting pancreatic lipase in order to reduce obesity like *Eleusine indica, Myristica fragrans, Melastoma candidum and Phyla nodiflora* (Ong *et al*., 2014) [26], *Citrullus lanatus* (Aruna *et al*., 2014) [27], *Abroma augusta* (Gupta *et al*., 2012) [28] etc.

Table 3: *In vitro* anti-obesity activity of *Gymnema sylvestre*

Samples	Concentrations (µg/ml)			
	100µg/ml	200µg/ml	300 µg/ml	400 µg/ml
Gymnema sylvestre (% of inhibition)	30.20±2.30	62.33±4.52	70.22±4.56	84.11±5.60
Standard (Orlistat)	26.54±1.8	58.75±3.20	79.65±4.02	92.55±6.32

Values are expressed as Mean ±SD triplicates

Conclusion

Overall, it can be concluded from the present study that *Gymnema sylvestre* leaves contains rich source of phytochemicals and possess anti-obesity activity. This study is the first scientific report that provides convincing phytochemicals and anti-obesity evidence for the relevance of *Gymnema sylvestre* leaves thus providing scientific validity to its traditional consumption by the local populace of south India.

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