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Evaluation of Antiobesity Activity Potential of Bioactive Constituents in Some Natural Plants

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Abstract: The aim of the present investigation involves the ethanolic extract of Alpinia calcarata rhizomes were subjected to phytochemical screening. The result indicated that, rhizome extract shows the presence of carbohydrate, proteins, cardiac glycosides, flavonoid, tannins and phenol. Phytochemicals are natural bioactive compounds found in plants. They are mainly two groups, which are primary and secondary metabolites. Primary metabolites are sugars, amino acids, proteins and chlorophyll while secondary metabolites consist of alkaloids, terpenoids and phenolic compounds. The activity of the ethanolic extract of Alpinia calcarata was orally bioscreened in progesterone-induced obese mice at 200 mg/kg/bw and 400 mg/kg/bw. Body mass index was calculated once per week for four weeks and blood samples were obtained at the end of the experiment for lipid profile analysis. Antiobesity activities of the rhizome extracts were compared with the controls. ethanolic extract of Alpinia calcarata rhizomes, at dose concentrations of 200 mg/kgbw and 400 mg/kgbw, showed significant effects on body mass index when compared to standard Semaglutide.

Keywords: *Alpinia calcarata rhizomes, obesity, invivo models, antiobsity.*

1. Introduction

Figure No.1: *Alpinia calcarata* – PLANT

Plant Description
Selected plant: *Alpinia calcarata*

Classification
Kingdom: Plantae
Division: Mangnoliphyta
Class: Liliopsida
Order: Zingiberales
Family: *Zingiberaceae*
Genus: Alpinia
Species: *Alpinia calcarata*

Synonyms
Alpinia calcarata Rosk., Alpinia erecta Lodd. and Steud., Alpinia bracheata Rosk., Alpinia cerrnta Sims., Renealmia calcarata Haw., Globba eracta Retx., Languascalcarata Mem.[1]

Selected Vernacular Names
Sinhala- Heen aratta, Aratta English- Galanga, Small galangaTamil- Amkolinji
Sanskrit- Rasna[1]

Distribution`
It is native to India. Occurs in Southern Malay Peninsula and Sri Lanka. It is common in village gardens in Sri Lanka [2]

Botanical description
Alpinia calcarata is a rhizomatous perennial herb with a non-tuberous rootstock, stems slender, about 75 cm tall; leaves simple, alternate, 25 - 32 cm long and 2.5 - 5 cm broad, lanceolate, acuminate, long-pointed, glabrous on both surfaces and shining on the upper surface, scantily hairy along the margin, petioles sheathing; flowers pinkish white, irregular, bisexual, in pendunculate, terminal, dense flowered panicles 8.5 cm long, two flowers together at each node, one opening earlier than the other, each bearing a pair of bracteoles, the inner one smaller than the outer, bracteoles oblong, papery white, each flower about 4 cm long, pedicels short, hairy; sepals 3, fused into a campanulate tube l cm long, pubescent outside, glabrous inside, apices rounded; petals 3, fused at base but segments free tinged with pink, segments oblong-spathulate, pubescent outside, lateral narrow;

staminodes 3, fused at base with the stamen into a tube adnate to corolla, two basal staminodes reduced to minute filaments, the larger one petaloid, 3 cm by 2.3 cm ovate, yellow with vinous red streaks, emarginated, apex frilled and darker, glabrous and shining on both surfaces; stamen, anther tubular, style passing through, filament flat, 1.5 cm long, anther 0.8 cm long, style 3.5 cm long, tinged pink, hairy towards the apex, stigma swollen; ovary inferior, 3 mm long, strongly pubescent, 3-locular with ovules in each loculus on a central axis capsules not seen[3, 4, 5].

Obesity is a serious problem in the world and has been associated with increase in morbidity, mortality, and reduced life expectancy [6]. It occurs as a result of energy imbalance between energy intake and energy expenditure, leading to increased lipid concentration in the blood and enlarged fat mass [7]. Although fat is vital for good health, buildup of a large amount of fat is linked to a variety of health risks such as dyslipidemia, diabetes mellitus, osteoarthritis, hypertension, fatty liver disease, cancers, asthma, and obesity [8, 9].

The prevalence of obesity is increasing rapidly worldwide. Presently, 300 million people are medically obese while more than one billion adults are overweight [10]. WHO also predicted that this number might increase to 3.3 billion by the year 2030. This disease has many factors which contribute to its etiology including sedentary lifestyle such as white collar jobs, lack of physical work out, increase in calorie consumption, endocrine disorders, and psychiatric issues among others [11, 12].

Previous studies also indicate that people increase their intake of high energy snack foods when stressed, thereby leading to obesity [13]. In addition, labor saving devices such as elevators, cars, remote controls, personal computers, and sedentary recreational activities such as watching television, browsing the Internet, and playing video games have highly contributed to obesity in the world [14, 15]. In spite of the urgent need for efficient and safe therapeutics and the probable size of the market for antiobesity drugs, the current efforts for improvement of such drugs are still unsatisfactory [16]. This is due to adverse side effects related to these drugs. More current approaches have focused on natural sources that have been reported to manage obesity and hyperlipidemia as well as reduce weight gain with fewer side effects [17]. Currently, potential use of natural agents for the management of obesity is not fully explored and could be an outstanding substitute approach for developing safe and effective antiobesity drugs hence hypothesis of present research made to develop potential anti-obesity with the help of ethanolic extract of *Alpinia calcarata* rhizomes.

Experimental work

Preparation of Methanolic Extracts

Ethanolic extract of *Alpinia calcarata* rhizomes was carried by soxlet apparatus.

Experimental Animals

A total of 45 female Swiss albino mice weighing an average of 23 g were used in this study. The animals were kept in cages under standard laboratory conditions (25 ± 2°C, 12 h light and 12 h dark cycle). They were then acclimatized to the environmental conditions for one week before the initiation of the experiment. Standard rodent pellets were used to feed the experimental animals and were supplied with water ad libitum.

Induction of Obesity

Obesity was induced in laboratory animals by subcutaneous administration of progesterone (DPMA) at a dose of 10 mg/kgbw. This was done daily, 30 minutes after oral administration of the extracts for 28 days except for the negative controls, which were not administered with the extract [18].

Antiobesity Assay

The mice of female sex were randomly divided into nine groups of five mice each and treated as follows: Group I (normal control) no treatment and Group II (negative control) depo-medroxyprogesterone acetate (DPMA) was administered subcutaneously in the dorsal neck region and the mice received oral administration of water (0.1 ml/mice). Group III (positive control) was given depo-medroxyprogesterone acetate (DPMA) (10 mg/kgbw) subcutaneously at the dorsal neck region and received standard drug orlistat (0.1 ml/mice); Groups IV and V (experimental groups) were given DPMA (10 mg/kgbw) and received Ethanolic extract of *Alpinia calcarata* rhizomes at dosages of 200 mg/kgbw and 400 mg/kgbw, respectively.

Determination of Body Mass Index

Animal weights and lengths (nasal-anal length) were monitored weekly for 4 weeks using an electronic precision balance and a ruler. To determine body mass index of mice, Lee index was used, which was defined asMice with BMI ≥ 310 were considered obese .

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$$BMI=(Bodyweight\sqrt{3}Nasal\text{-}anal\ length\ (cm))\times 1000. \quad (1)$$
Mice with BMI ≥ 310 were considered obese

Blood Collection and Sera Samples Preparation
At the end of the experimental period (on the 29th day), the tail of each mouse was nipped and venous blood from the tail was collected. Blood glucose test was carried out using a glucose analyzer model (Hypogaurd, Woodbridge, England). The mice were then euthanized using chloroform to minimize stress and pain during sacrificing. The mice were laid on a dissecting board using pins on a bench and then sacrificed. Blood samples were then collected by cardiac puncture and transferred into plain microvacutainer tubes immediately.

The blood samples were then centrifuged at 2400 rpm for 10 minutes to collect clear serum. The clear serum was then aspirated off, packed in Eppendorf tubes and stored frozen at −20°C awaiting analysis. Olympus 640 chemistry auto analyzer was used for analysis of lipid profiles (TG, TC, HDL-C, and LDL-C). All assays were performed based on the standard operating procedures (SOPs) written and maintained at the Department of Biochemistry, Thika Levels Hospital.

Qualitative Phytochemical Screening
The crude extracts were subjected to qualitative phytochemical screening to identify presence or absence of selected bioactive compounds using standard methods Secondary metabolites tested included alkaloids, terpenoids, diterpenes, flavonoids, phenolics, saponins, anthraquinones, steroids, and tannins.

Saponins (Froth Test). One gram (1 g) of each plant extract was separately added in 2 ml of distilled water in a test tube, sodium bicarbonate solution was added drop wise, and the mixture shaken vigorously. The occurrence of frothing which persisted for at least 15–20 minutes indicated saponins presence.

Alkaloids. One gram (1 g) of each plant extract was separately added to 2 ml of 1 molar aqueous concentrated hydrochloric acid. The mixture was stirred and heated in a water bath for 5 minutes and then cooled. Thereafter, the mixture was filtered with Whitman's filter paper number 1 and two drops of Dragendorff's reagent were added. A color change to orange after addition of Dragendorff's reagent indicated presence of alkaloids.

Terpenoids (Salkowski Test). One gram (1 g) of each plant extract was separately added to 1 ml of ethyl acetate/petroleum ether and mixed into 2 ml of chloroform. Three milliliters (3 ml) of concentrated sulphuric acid was added alongside to form a layer. A reddish brown coloration of the interface was formed to show presence of terpenoids.

Phenols. The crude extracts were screened for phenols by adding 1 ml of ferric chloride solution to 1 g of each plant extract. Formation of blue to green color indicated the presence of phenols.
Tannins. One milliliter (1 ml) of distilled water was added to each plant extract followed by two drops of 5% iron chloride. Blue-black coloration indicated presence of tannins.

2. Results

The mice of female sex were randomly divided into nine groups of five mice each and treated as follows: Group I (normal control) no treatment and Group II (negative control) depo-medroxyprogesterone acetate (DPMA) was administered subcutaneously in the dorsal neck region and the mice received oral administration of water (0.1 ml/mice). Group III (positive control) was given depo-medroxyprogesterone acetate (DPMA) (10 mg/kgbw) subcutaneously at the dorsal neck region and received standard drug orlistat (0.1 ml/mice); Groups IV and V (experimental groups) were given DPMA (10 mg/kgbw) and received Ethanolic extract of *Alpinia calcarata* rhizomes at dosages of 200 mg/kgbw and 400 mg/kgbw, respectively.

Table No: 1 Treatment and number of mice used rhizomes Body Antiobesity Activities of Ethanolic extract of *Alpinia calcarata* rhizomes on Body Mass Index (BMI) in Progesterone-Induced Obese Mice

Treatment groups	Treatment	Number of mice
I (Normal group)	Water ad libitum	5
II (Negative control)	Depo-medroxyprogesterone acetate (DMPA) (10 mg/kgbw)	5
III (Positive control)	DMPA (10 mgkgbw) + (Semaglutide.) (10 mg/kgbw)	5
IV	DMPA (10 mg/kgbw) + extracts (200 mg/kgbw)	5
V	DMPA (10 mg/kgbw) + extracts (400 mg/kgbw)	5

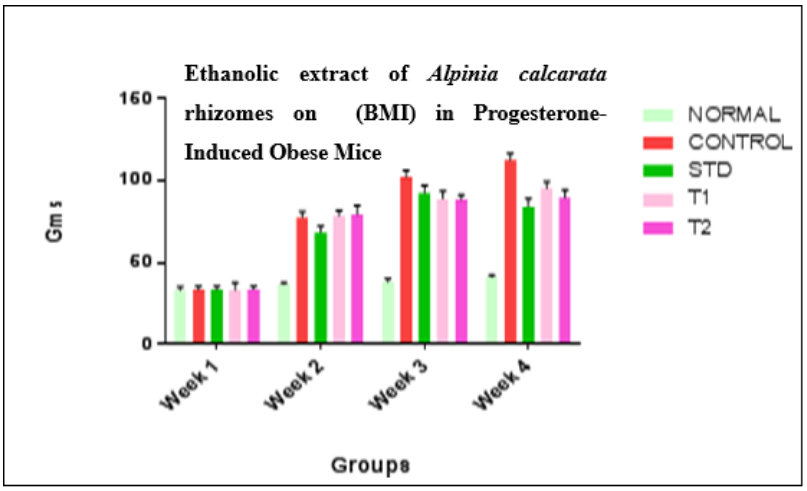
The Ethanolic extract of *Alpinia calcarata* rhizomes caused changes in the body mass index (BMI) of progesterone-induced obese mice (Table 2). The body mass index of mice administered with the extract at a dose of 200 mg/kgbw had a similar trend with that of normal control (placebo). The BMI for the mice treated with extract dose of 200 mg/kgbw and normal control were insignificantly different at days 7, 14, 21, and 28 ($p > 0.05$; Table 2). Moreover, mice treated with extract dose of 200 mg/kgbw had a significant decrease in body mass index compared to the mice treated with progesterone (negative control) at days 7, 14, 21, and 28 ($p < 0.05$; Table 2). Mice administered with extract dose of 200 mg/kg/bw had a comparable increase in body mass index with mice treated with orlistat (positive control) at day 7 ($p > 0.05$; Table 2). However, mice treated with 200 mg/kgbw dose of the extract had a significant increase in body

Table No: 2 Antiobesity Activities of Ethanolic extract of *Alpinia calcarata* rhizomes on Body Mass Index (BMI) in Progesterone-Induced Obese Mice

Treatment	Time (Days)				
	0	**7**	**14**	**21**	**28**
Normal control	296.57 ± 2.01[a]	306.348 ± 1.95[b]	307.41 ± 1.64[b]	308.72 ± 1.10[b]	309.97 ± 0.76[b]
Progesterone (10 mg/kg bw)	308.41 ± 1.48[a]	316.48 ± 2.34[a]	314.97 ± 1.94[a]	318.41 ± 1.84[a]	319.49 ± 1.79[a]
Semaglutide.) (10 mg/kg bw)	305.16 ± 1.02[a]	297.70 ± 2.06[b]	296.49 ± 1.91[c]	292.48 ± 1.69[c]	289.40 ± 1.97[c]
Ethanolic extract of Alpinia calcarata rhizomes (200 mg/kgbw)	298.84 ± 0.91[a]	301.69 ± 0.56[b]	304.53 ± 0.99[b]	304.53 ± 2.66[b]	306.89 ± 1.99[b]
	300.84	295.34	290.60	290.90	289.11

Ethanolic extract of Alpinia calcarata rhizomes (400 mg/kgbw)	± 1.13[a]	± 2.93[b]	± 2.49[c]	± 3.35[c]	± 2.44[c]

Figure No.2 Graphical representation of extracts compared with control and standard drug



Where T1= Ethanolic extract of Alpinia calcarata rhizomes (200 mg/kgbw)
T2= Ethanolic extract of Alpinia calcarata rhizomes (400 mg/kgbw)

3. Discussion

Obesity is defined as a chronic metabolic disorder that is characterized by increased lipid concentration and enlarged fat mass. It is a result of imbalance between energy expended and energy taken in [18]. From the results it is revealed that the extracts shown potent antiobesity activity at 200mg/bw and 400mg/bw when compared to the standard Semaglutide

4. Conclusion

From the present research work it was found that the Ethanolic extract of Alpinia calcarata have antiobesity activity and reveal the presence of vital phytochemicals like alkaloids tannins, phenols cardiac glycosides. The antiobesity activity of the studied plant may have resulted from its phytochemicals constituents. It provided evidence that these extracts decrease body mass index.

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