

**Title: Beneficial Effects of Natural Bioactives of *Hibiscus Sabdariffa* on Obesity**

Oyindamola Vivian Ojulari<sup>a</sup>, Seul Gi Lee<sup>a</sup>, Ju-Ock Nam<sup>a,b\*</sup>

<sup>a</sup>Department of Food Science and Biotechnology, Kyungpook National University, Daegu, 41566, Republic of Korea

<sup>b</sup>Institute of Agricultural Science & Technology, Kyungpook National University, Daegu, 41566, Republic of Korea

**\*Corresponding author.**

Department of Food Science and Biotechnology, Kyungpook National University, Daegu, 41566, Republic of Korea. E-mail address: namjo@knu.ac.kr (J.-O. Nam).

**Abstract:** Obesity is a condition associated with the accumulation of excess fat in the body, energy imbalance, lipogenesis etc. which increases adipose tissue mass through adipogenesis and probes a health risk. Its prevalence has become a large burden on the world at large. One of the solutions to tackling obesity is with the use of bioactive compounds. We critically examined the effectiveness of *hibiscus sabdariffa* (HS) on various parameters associated with development of obesity such as; effect of HSE on body weight and energy expenditure, effect of HSE on fat accumulation, effect of HSE on lipase inhibition, effect of HSE on adipocyte

differentiation/adipogenesis. This review has gathered reports on the various anti-obesity effects of HS bioactives in cell and animal models, as well as in humans. Reports have shown that *hibiscus sabdariffa* derived bioactives are potent in the treatment of obesity with evident reduction in body weight, inhibition of lipid accumulation and suppression of adipogenesis through PPAR $\gamma$  pathway and other transcriptional factors.

**Keywords:** Adipogenesis, bioactive compounds, fat accumulation, *hibiscus sabdariffa*, lipase inhibition

### Abbreviations

Adipose tissue	AT
Epigallocatechin gallate	EGCG
Free fatty acid	FFA
High-density lipoproteins	HDL
<i>Hibiscus sabdariffa</i>	HS
<i>Hibiscus sabdariffa</i> extract	HSE
Interleukin-1	IL-1
Low-density lipoproteins	LDL
Messenger RNA	mRNA
Metabolic syndrome	MS

Not specified	NS
Peroxisome Proliferator-Activated Receptor Gamma	PPAR $\gamma$
Sterol regulatory element-binding protein	SREBP-1c
Tumor necrosis factor alpha	TNF- $\alpha$

## 1. Introduction

Obesity is a major public health problem associated with an increased incidence of metabolic diseases like type 2 diabetes, high blood pressure, heart disease, liver disease, kidney disease, gallbladder disease, and certain types of cancer [1]. The underlying cause of obesity can be attributed to the dietary pattern, genetic disorders, sedentary lifestyle, and psychological factors. The imbalance between energy intake and expenditure also cause the buildup of excess adipose tissue [2].

Several approaches have been made towards combating obesity which includes the clinical approach like exercising to induce weight loss and reduce fat accumulation, dietary approach like the caloric restriction, ketogenic diet and the development of drugs. However, this approach has been found to be seasonal and not long-lasting [3] as it eventually gives a *yo-yo* effect. For a more lasting solution, research is leaning towards the use of bioactive compounds in plant materials as a novel therapy to combat obesity and its related diseases.

Recently, natural bioactives such as flavonoids and phenols have been reported to be effective in the treatment of obesity [4-7]. Bioactive compounds in edible plants such as epigallocatechin gallate (EGCG) from green tea, nobiletin from citrus peel, curcumin from turmeric, resveratrol, pterostilbene from berries and anthocyanins from blueberries and *hibiscus sabdariffa* have been reported for their anti-obesity potential in both in-vivo and in-vitro studies [8-12]. Among these medicinal plants, *Hibiscus sabdariffa* (HS) also known as Roselle has a long history of its use as beverage and folk medicine in Asia, Africa, China, India, Thailand, Nigeria [13-15].

Studies on the phytochemical properties of HS showed that it has several health benefits and could be used as a potent material for therapeutic treatment of various diseases [16, 17]. Its

chemical composition also showed that the therapeutic potency of *H. sabdariffa* could be traced to the presence of bioactive compounds. Bioactives such as flavonoids (quercetin, luteolin, and its glycoside); chlorogenic acid, gossypetin, hibiscetin, phenols, some phenolic acids, anthocyanins such as delphinidin-3-sambubioside and cyanidin-3-sambubioside were detected as the main components in the aqueous extract of *H. sabdariffa* [18]. These bioactives together or alone has been reported in many studies to possess powerful anti-obesity, antioxidant and anti-inflammatory activities, anti-carcinogenic effects, and may also help prevent cardiovascular disease and control diabetes [19-26].

Although many report are available on the effect of HS in the treatment and management of obesity and related metabolic diseases. However, a review on *hibiscus sabdariffa* and its bioactives in relation to obesity are still insufficient. Most reviews have focused on the phytochemical, pharmacological and toxicological properties of HS [13, 15]. Other comprehensive reviews documented the effect of *hibiscus sabdariffa* the treatment of hypertension hyperlipidemia and apoptosis [27-30]. Its traditional use, nutritional composition, bioactive constituents, health benefits and therapeutic use was examined by [17, 31]. A review by [32] focused on the multi-targeted molecular effect of hibiscus polyphenols on obesity management. Therefore, this present review takes into consideration available reports on the beneficial effects of *hibiscus sabdariffa* bioactive compounds in obesity therapy.

## **2. Natural bio actives in *hibiscus sabdariffa***

Bioactive compounds are compounds produced by plants having pharmacological or toxicological effects in man and animals [33]. Various natural compounds have been identified to influence weight loss, fat accumulation, and avert diet-induced obesity. Hence, these products

have been extensively consumed for the treatment of abdominal obesity and overweight [4, 34, 35].

As seen in Table 1, HS is an important source of bioactive compounds, including anthocyanins, flavonoids, phenolic acids and organic acids.

**Table 1 Major bioactive compound in *H. sabdariffa***

<b>Bioactives</b>	<b>References</b>
<b>Organic acids</b>	[37, 65-68]
Hydroxycitric acid	
Hibiscus acid	
Dimethyl hibiscus acid	
<b>Anthocyanins</b>	[32, 69]
Delphinidin- 3-sambubioside (hibiscin)	
Cyanidin-3-sambubioside (gossypicyanin)	
Cyanidin-3,5-diglucoside	
Delphinidin (anthocyanidin)	
<b>Flavonoids</b>	[66, 70]
Hibiscitrin (hibiscetin-3-glucoside),	
Sabdaritrin	
Gossypitrin	
Gossytrin	

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Gossypetin glucosides

Quercetin

Luteolin

**Phenolic acid**

[37, 66, 69]

Chlorogenic acid

Protocatechuic acid

Ellagic acid

P-Coumaric acid

Ferulic acid

Caffeic

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Different studies (as shown in Table 2) have attributed the anti-obesogenic potential of HSE in obesity treatment to one or more specific compounds in HSE [11, 36]. While some authors reported polyphenols [8, 20, 37] and anthocyanin [9, 10] as the main active component in HS, another group of authors concluded in their report that other organic acids (hibiscus, dimethyl hibiscus and hydroxycitric acid) contained in HS were responsible for the beneficial effects observed in their study [36]. Various reports on the effects of these compounds on obesity treatment will be highlighted under different sections of this review.

**Table 2 Studies on *H. sabdariffa* bioactives and their anti-obesity effects**

Bioactive compound	Extraction solvent	Effective Dose	Type of study	Observed effect(s)	References
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NS	Aqueous	500, 1000 mg/kg	In vivo (Sprague-Dawley rats)	Significantly decreased serum cholesterol, triglycerides and LDL levels	[26]
Anthocyanin	Aqueous	1 g HSE capsule-dose	In vivo (human trial)	Decreased serum FFA level of the HSE group and improved liver steatosis	[48]
Polyphenols	Methanol	100 mg/kg, 200 mg/kg	In vivo (rat; animal model)	Significantly reduced the plasma level of triacylglycerol cholesterol, and LDL/HDL risk ratio	[66]
Polyphenols	Methanol	200 mg/kg	In vivo (rat; animal model)	HPE inhibited fat deposition, hyperglycemia, serum advanced glycation end-products (AGE)	[71]
Anthocyanin	Aqueous	33.64 mg/kg	In vivo (mice)	Suppressed body weight gain in Ob/MSG mice by 9.6% and reduces glycaemia	[9]
Anthocyanin	Aqueous	33 mg/kg	In vivo C57BL/6NHsd mice	Reduced fat tissue accumulation, normalized the glycemic index as well as reduced dyslipidemia	[40]

Polyphenols	Aqueous	1% and 2%	In vivo Syrian hamsters	Triglycerides levels, fatty acid concentrations and liver cholesterol were decreased in a dose-dependent manner	[8]
Polyphenols	Methanol	0.25 or 0.5 mg ml <sup>-1</sup>	3T3-L1 pre-adipocytes cell	Suppressed differentiation and increased number of apoptotic cells in mature adipocytes	[8]
NS	Aqueous	2mg/ml	3T3-L1 pre-adipocytes cell	Inhibition of lipid accumulation by reducing intracellular lipid droplet during adipogenesis	[10]
Hibiscus, Dimethyl hibiscus, and Hydroxycitric acid	Aqueous and Methanol	3% of 750mg (22.5mg)	In vivo (wistar male rats)	Prevented increase in body weight and decreased adipocytes hyperplasia on rats fed a hypercaloric diet	[36]
Anthocyanin	Aqueous	33mg/kg	In vivo (C5BL/6NHsdmice)	Reduced body weight gain, fat accumulation, normalized glycemic index and reduced dyslipidemia	[40]
NS	Ethanol	5%, 10%, 15%	In vivo in rats	Prevented increase in body weight,	[12]

				triglyceride, LDL and total lipids were lowered	
NS	Aqueous	0.5% and 1%	In vivo rabbits	Decreased cholesterol, LDL and triglyceride to near normal values	[72]
Polyphenols		100mg (Capsule)	In-vivo (clinical study)	Patients with metabolic syndrome showed a significant reduction in total cholesterol and glucose level, with increased HDL level.	[47]
NS	Aqueous	100 mg/ml	3T3-L1 pre-adipocytes cell	Reduced the expression of major adipogenic transcription factors including PPAR $\gamma$ and C/EBP $\alpha$ that regulate adipogenesis	[64]
Polyphenols and Anthocyanin	Aqueous	100 mg/kg	In-vivo male hamsters	Decreased the levels of serum and hepatic lipids such as total cholesterol and triglyceride in HFD-fed hamsters	[11]

Polyphenols	Aqueous and ethanol	500 µg/ml aqueous extract and ethanol extract 10 µg/ml	3T3-L1 pre-adipocytes	Inhibited lipid accumulation and adipogenic differentiation of pre-adipocytes	[37]
Anthocyanin	Aqueous	100 mg/kg	Male Sprague-Dawley rats	Attenuated body weight gain, plasma leptin, cholesterol, triglycerol, LDL and systolic blood pressure	[73]
NS	Aqueous	500 mg/kg	In vivo ( Sprague Dawley rats)	Prevented the hypercholesterolemia induced by dietary fructose	[74]

### 3. Effect of *Hibiscus sabdariffa* extract (HSE) on obesity

#### 3.1. Effect of HSE on body weight and energy expenditure

As aforementioned, obesity can be characterized by excessive energy intake and lack of energy expenditure [2, 38]. This disruption in energy homeostasis results in abnormal adipocyte differentiation, which is characterized by hyperplasia and hypertrophy of adipocytes [39]. The potentiality of polyphenols in *hibiscus sabdariffa* to regulate energy metabolism and its beneficial effect on lipid management and weight loss as studied by several authors will be examined in this section.

According to Morales-Luna et al., [36], it was reported that 22.5mg aqueous HSE of the white variety of Roselle prevented the increase in body weight of rats fed a high-fat fructose diet.

Villalpando-Arteaga et al. [40] also reported a reduction in body weight in relation to inhibition of fat accumulation of obese C57BL/6NHsd mice after treating with 33 mg/kg three times a week for 8 weeks. Impressively, Alarcon- Aguilar et al., [9] recorded a 9.6% decrease in body weight of obese MSG mice with daily administration of 33.64 mg/kg/day for 8 weeks. A significant effect in body weight was noticed from the 7th week, which was attributed to a reduction in food with a consequent increase in liquid intake.

Herrera-Arellano et al., [25] who also reported a similar observation attributed this result to the diuretic effect of HS. In spite of these observations, the author suggested that further long-term toxicity studies should be performed on this plant, especially since the previous study by Akindahunsi and Olaleye [41] have identified in their study that prolonged usage of this extract at 15-dose level caused liver injury while the effect was mild at small dose levels (1–10). Carvajal-Zarrabal et al., [12] also reported that HSE administered at concentrations of 5 to 15% was effective in body weight reduction at intermediate and greater concentrations of 10 and 15% used in their experiment. In vitro and in vivo studies showed that Hibiscus extract (or tea) inhibited the activity of  $\alpha$ -amylase, blocking sugars and starch absorption, which may assist in weight loss [42, 43]. Overall, most of these studies attributed the ability of HSE in reducing body weight to HS polyphenols and flavonoids, through the inhibition of fat accumulation [9, 40].

### **3.2. Effect of HSE on Fat Accumulation**

Apart from other factors liable to cause the onset of obesity. The major mark of obesity is the abnormal or excessive fat accumulation in adipose tissue. The amount of excess fat in absolute terms, and its distribution in the body - either around the waist and trunk (abdominal,

central or android obesity) or peripherally around the body (gynoid obesity) have important health implications [44]. The effect of HS on weight loss in early studies prompted further studies on its effect on lipid profile. Quite a number of studies have emphasized HSE to have an effect on inhibiting and/or reduce fat accumulation.

Bioactive compounds (polyphenolic and flavonoids) in HS have been reported to decrease oxysterols (a derivative cholesterol) in bile acid metabolism and block lipid accumulation in the liver [45]. In a study conducted by Carvajal et al., [12] on the modulation of fat absorption in rats by HSE extract, it was reported that HSE modulated fat absorption by increasing palmitic acid excretion in faeces, accompanied by a decrease in triglycerides and cholesterol levels, including low-density lipoproteins (LDL) cholesterol. Another study carried out to evaluate the effects of HSE extract powder on the lipid profiles of individuals with and without metabolic syndrome (MS) showed that HSE significantly reduces glucose and total cholesterol levels; increased HDL levels and triglycerides/HDL ratio in patients with metabolic syndrome (MS) [46]. In cholesterol-fed rabbits and high fructose-fed rats, HSE extract also decreased the number of oxidized LDL positive foam cells and total cholesterol and triglycerides concentrations in plasma [47].

A more recent study investigated the effect of HSE in the reduction of fat tissue accumulation in high fat diet-induced obese C57BL/6NHsd mice [40]. A high-fat diet (HFD), alongside with reduced physical activity, induces excessive storage of triglycerides in adipocytes that leads to hypertrophy of the adipose tissue (AT). The study reported that HSE greatly diminished the accumulation of fat in the cytoplasm of hepatocytes. A significant reduction ( $p < 0.05$ ) was observed in the gene expression of both transcription factors PPAR $\gamma$  and SREBP-1c in obese mice supplemented with HSE compared to obese mice. These authors claimed that HSE

extract regulated the lipid homeostasis through SREBP-1c and PPAR $\gamma$  inhibition by blocking the increase of IL-1, TNF- $\alpha$  mRNA and lipoperoxidation and increased catalase mRNA; counteracting liver damage in an agonist-dependent manner. Hence, they concluded that Hs extract possesses an anti-steatogenic effect in the liver besides the anti-lipidemic and anti-obesogenic effects in the HFD-induced obese mouse model. Considering that fat accumulation is highly associated with obesity, accumulations of fat in organs have been a major concern in obesity management.

In liver steatosis, the anti-steatogenic effect of HSE on fatty liver (caused by fat accumulation in the liver) was recently conducted on humans. A clinical study conducted on patients with fatty liver within the age of 18-65 revealed that 2 HSE capsule-dose (1g) after meals, 3 times a day significantly reduce the level of serum free fatty acid (FFA), exerting a beneficial effect on metabolic regulation, while improving the liver steatosis. However, this study observed no significant difference in the lipid profile except for FFA [48]. Furthermore, it was inferred in this study that polyphenol was mainly responsible for the clinical effect of HSE and hence an increase in HSE dose could be more effective. A dose-dependent decrease in triglycerides levels, fatty acid concentrations and cholesterol contents of plasma lipids and liver lipids were observed in an in-vivo study of high-fat induced male Syrian hamsters fed with HSE [8]. HSE effectively inhibited lipid accumulation from fat-feeding and decrease the cholesterol in plasma and organs (liver). Affirmatively, these authors also attributed the effects observed in HSE-fed hamsters to the presence of polyphenols in the HSE. Clinical studies on patients with metabolic syndrome, an obesity-associated disorder further supported previous reports that polyphenols may be responsible for the therapeutic effect in HS extract [20].

Based on these studies, it can be inferred that the presence of natural bioactive compounds such as polyphenols, flavonoids, and organic acids in HSE could be used as a preventive therapy in combating fat-induced obesity.

### **3.3. *Effect of HSE on Lipase Inhibition***

Another strategy that has been proposed for the treatment of obesity is to inhibit pancreatic lipase which consequently decreases lipid absorption in the intestine [49]. The underlying concept is that for any dietary fat being absorbed in the human intestine, the fat should be broken down enzymatically by the action of pancreatic lipase [50]. Pancreatic lipase activity is therefore widely considered as one of the most important indicators for the determination of the anti-obesity potential of natural products [51]. Orlistat, a potent, specific, irreversible inhibitor of pancreatic and gastric lipases is a weight-loss agent with a novel mechanism of action for the treatment of obesity. It inhibits gastric and pancreatic lipases in the lumen of the gastrointestinal tract to decrease systemic absorption (30%) of dietary fat [52]. However side effects such as diarrhea, fecal incontinence, flatulence, bloating and dyspepsia are commonly developed [11]. Due to these adverse effects, it has been a long-standing interest in discovering well-tolerated natural inhibitors for nutrient digestion and absorption.

The potential inhibitory activity against pancreatic lipase was also reported by examining the effect of HSE extracts on fat absorption-excretion and body weight in rats [12]. Thus, continuous administration of HS polyphenols might improve obesity-related metabolic disorders in a similar manner to orlistat [32]. Although this action may be viewed as a potential strategy in obesity management, however, its mechanism in obesity therapy is yet to be explored.

### 3.4. *Effect of HSE on adipocyte differentiation (Adipogenesis)*

Adipogenesis is the process by which cells differentiate into adipocytes. This process involves the conversion of preadipocytes into mature adipocytes with intracellular lipid accumulation [53]. Adipocytes are cells that primarily compose adipose tissue, specialized in storing energy as fat [54]. They play an important role in regulating adipokine secretion which promotes adipogenesis. Therefore, understanding the molecular mechanisms that regulate adipogenesis is important for exploring anti-obesity therapy [55].

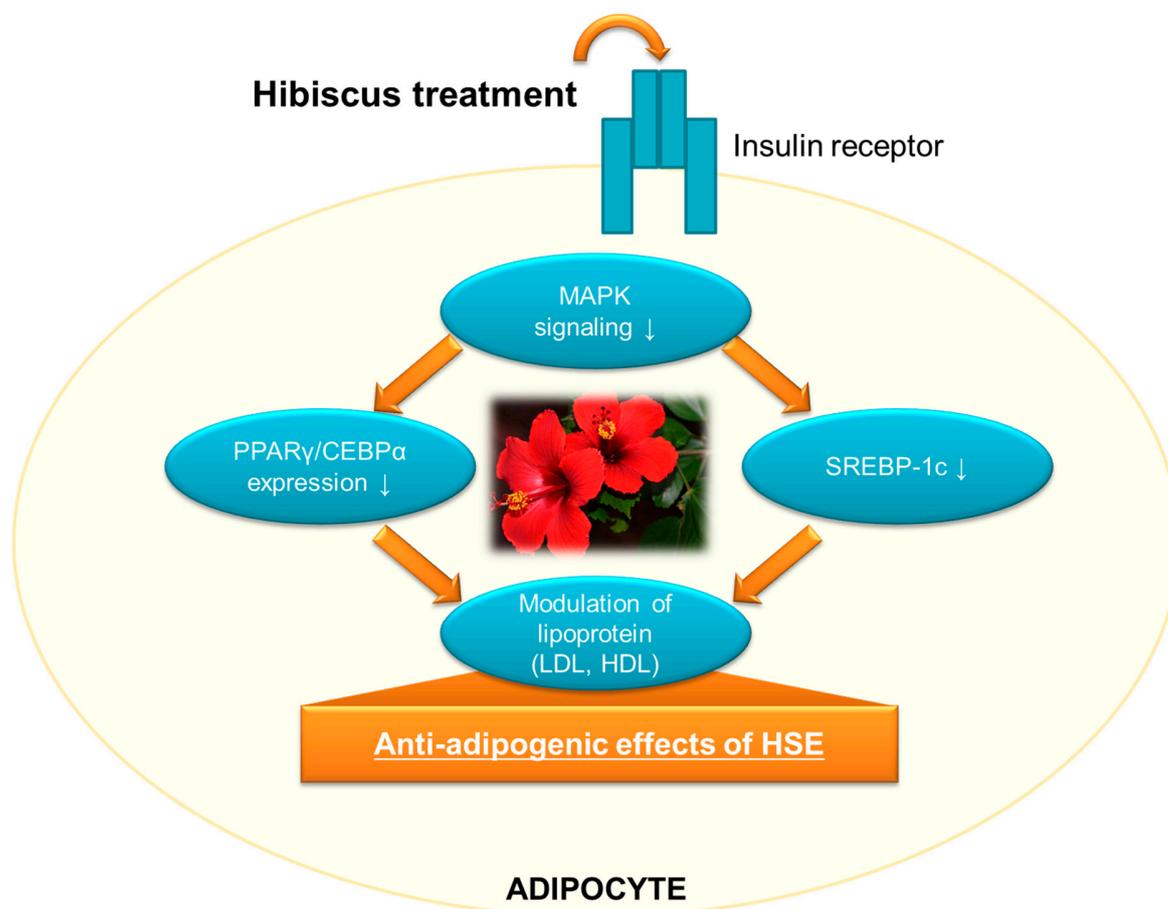
Adipocyte differentiation is a critical phenomenon in the development and progression of obesity [56]. Adipocyte differentiation has been reported to be mainly mediated by the transcription factors PPAR $\gamma$  and C/EBP $\alpha$  [53]. These adipogenic transcription factors that are implicated to activate a number of genes induced during adipocyte differentiation is a master regulator of adipogenesis [57-59]. Hence, a down-regulation of PPAR $\gamma$  and C/EBP $\alpha$  has been viewed as a strategy to obstruct adipogenesis in adipocytes. Several studies have reported that extract of various medicinal plants attenuates expression of PPAR $\gamma$  and C/EBP $\alpha$  [10, 40, 60-63].

So far only a few studies have reported and confirmed the effect of HSE on adipocyte differentiation. Kim et al., [64] first reported the effect of HSE treatment on adipocyte differentiation from 3T3-L1 preadipocytes and found that HSE blocked adipogenesis, possibly mediated through the suppression of adipogenic transcription factor expression. HSE treatment (100 mg/mL) inhibited the expression of major adipogenic transcription factor PPAR- $\gamma$  and C/EBP- $\alpha$ , nuclear hormone receptors that regulates adipogenesis during differentiation. The authors further stated that this inhibitory effect of HSE on the transcription factors was target specific [64]. Further studies by Kim et al. [10], also confirmed that HSE can inhibit the adipogenic transcription factors by blocking the MAPK-mediated signaling pathway during

adipocyte differentiation. They reported that HSE significantly decreased the mRNA levels of Leptin during differentiation. Hence, suggesting that the effect of hibiscus extract on adipocyte differentiation was also mediated by the regulation of Leptin.

A recent study performed on 3T3-L1 pre-adipocytes cells revealed that HSE aqueous extract and HS polyphenols at concentrations 500µg/ml and 10µg/ml significantly inhibited adipogenic differentiation of pre-adipocytes [37]. These authors concluded that polyphenols contained in *hibiscus sabdariffa* are mainly accountable for its effect on adipogenesis. Kao et al. [8], also supported that HS polyphenolic extract (HPE) was more efficient in suppressing adipogenesis than HS extract (HSE) as markers of adipocyte differentiation, such as SREBP 1 were found to decrease in a concentration-dependent manner following treatment with both HSE and HPE. The authors reported in their study that after inducing maturation of preadipocyte, HPE suppressed the adipogenesis of mature adipocyte cells which corroborates with the previously reported findings that polyphenols are the major active components in HSE responsible for its anti-obesogenic effect.

Based on the literatures reviewed, *hibiscus sabdariffa*'s natural bioactive such as polyphenols have shown good potential to modulate PPAR $\gamma$  and other transcriptional activities as shown in fig 1, which makes it a potential therapy to inhibit adipogenesis and hence, combat obesity.



**Figure 1. Anti-adipogenic effects on HSE through different pathways**

#### 4. Conclusion

Obesity is a common but often underrated condition that has become a big social issue in so many different countries around the world and contributes significantly to the rate of morbidity and death. The cause of obesity has many faces and several methods have been used to approach the decline of this disease. From the reviewed studies, it is evident that the presence of bioactives in *H. Sabdariffa* enacts it with potential use for pharmaceutical applications. However, inconsistency of available studies about the major compound responsible for anti-obesogenic

properties of *H. sabdariffa* makes necessary to carry out further studies. Since many clinical and animal studies have reported HS bioactives to be effective in combating obesity, its use as an active ingredient incorporated into diets is suggested. Notwithstanding, standard extraction protocols for specific bioactives as well as an effective dosage for the treatment of obesity should be developed for a more unanimous report.

### **Conflicts of interest**

The authors declare that there are no conflicts of interest.

### **Author's contributions**

Conceptualization, O.V.O, S.G.L and J.O.N; Writing-original draft, O.V.O; Review & Editing, O.V.O and S.G.L; Supervision, J.O.N. All authors have read the manuscript before submission.

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