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EVALUATION OF ANTIOBESITY ACTIVITY OF JUSTICIA TRANQUEBARIENSIS ON PROGESTERONE-INDUCED OBESE RATS

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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, anti oxidant and anti-obesity activity of *Justicia tranquebariensis* extract. The phytochemical screening *Justicia tranquebariensis* showed that the presence of saponins, flavonoids, steroids, terpenoids, polyphenol, and alkaloids in ether, ethanol and aqueous extracts. Quantitative analysis of phenol and flavonoids was carried out. Significant amount of flavonoids and phenols were present. The anti-obesity activity of *Justicia tranquebariensis* was proved by inhibition of lipase. Overall, it was concluded from the present study that *Justicia tranquebariensis* extract possesed rich source of phytochemicals and hence anti-obesity activity.

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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 [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 [2]. 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 *Justicia tranquebariensis* extract.

Materials and Methods

Collection of plant material

The plant powder of *Justicia tranquebariensis* was collected from Chittoor District, Andhra Pradesh, India.

Preparation of plant extract

The dried leaves were ground into powder and sieved. The obtained powder was stored in a tightly closed amber-colored bottle. In the leaf, the chlorophyll was removed by standard method (Pet. ether). Six glass bottles were taken and 200ml each solvent such as Hexane, Ethyl acetate, Chloroform, pet ether, Ethanol, and Distilled water was added. To each bottle, 100g of the plant powder was added and soaked for 24 hrs. The mixtures were then filtered and the filtrates were subjected to evaporation using a water bath to remove the solvents. Percentage yield was calculated for each solvent and the results are shown in Table 1.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Trease and Evans (1989) and Harborne (1973) [3, 4].

Determination of total polyphenol content

The polyphenol content was determined according to the method of Singleton et al. (1999) with some adjustments [5]. The Folin-Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate with a yellow color. It is used for the quantification of compounds containing phenols or polyphenols. In this experiment, the reaction mixture consisting of 250 µL of extract (or standard), 250 µL of water, and 250 µL of Folin Ciocalteu reagent was mixed well. Na2 CO3 10% was added and incubated for 30 min at 40 ºC in a thermostat. The spectral absorbance of the reaction mixture was measured at 765 nm. The total polyphenol content in the extract was determined based on the standard curve equation of gallic acid y = 0.0403x - 0.0033 with the coefficient R2 = 0.9988 (where: y-axis referred to the spectral absorbance value (Abs), the x-axis corresponds to gallic acid standard concentration).

Determination of total flavonoid content

Total flavonoid content was determined according to the description of Bag et al. (2015) [6]. The reaction mixture consisted of 200 µL of extract or standard, 200 µL of water, and 40 µL of NaNO2 5% was shaken and allowed to stand for 5 min. Then, 40 µL of AlCl3 10% was added to the mixture and shaken well. After incubation for 6 min, the reaction mixture was added 400 µL of 1 M NaOH and water to make 1 mL. The reaction mixture was measured absorbance spectrophotometrically at 510 nm. The total flavonoid content in the extract was determined based on the standard curve equation of quercetin y = 0.006x – 0.0235 with the coefficient R2 = 0.9985 (where: the y-axis referred to the value of spectral absorbance (Abs), the x-axis referred to the concentration of the quercetin standard).

In vitro antioxidant assays

DPPH free-radical scavenging activity

The free radical scavenging activity of the plant extract was measured using DPPH by the method of Kumarasamy et al. [7] 80 µg/ml DPPH was prepared with methanol. Serial dilutions were carried out with the 1 mg/ml stock solutions of the extracts. 2 ml of each solution was then mixed with 2 ml of DPPH and allowed to stand for 30 minutes; the absorbance was then read at 517 nm. Ascorbic acid was used as standard. IC50 value was also calculated using a concentration-response curve.

Inhibition of DPPH free radical in percentage was calculated by formula:

DPPH radical scavenging activity (%) = (A control-A test)/A control×100

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging potential of the plant extract was determined using the method described by Jayaprakasha et al. [8]. A solution of hydrogen peroxide (20 mM) was prepared in (phosphate buffer saline [PBS], pH 7.4). Different concentrations of the extract (20-100 µg/ml) in ethanol (1 ml) were added to 2 ml of hydrogen peroxide solution in PBS. After 10 minutes the absorbance was measured at 230 nm against blank solution that contained hydrogen peroxide solution without the extract.

The percentage of H_2O_2 scavenging of the plant extract was calculated as follows:

% Scavenged [H_2O_2] = [(Abs control - Abs sample)/Abs control] ×100

Nitric oxide radical (NO) scavenging assay

Nitric oxide (NO) generated from sodium nitroprusside (SNP) was measured according to the method of Marcocci et al., 1994 [9]. Briefly, the reaction mixture (5.0ml) containing SNP (5mM) in phosphate buffered saline (pH 7.3), with or without the seed extract at different concentrations, was incubated at 25°C for 180min in front of a visible polychromatic light source (25 Watt tungsten lamp). The NO radical thus generated interacted with oxygen to produce the nitrite ion (NO^{2-}) which was assayed at 30 min intervals by mixing 1.0 ml of incubation mixture with an equal amount of Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% Nnaphthylethylenediamine dihydrochloride). The absorbance of the chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulphanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was measured at 546nm. The nitrite generated in the presence or absence of the seed extract was estimated using a standard curve based on sodium nitrite solutions of known concentrations.

Anti-obesity activity of Ethanolic extract of *Justicia tranquebariensis* on Progesterone-induced Obesity in rats

Female albino rats were used for the study. 6-10 weeks old, 250-350g rats were used. All animal experiments were undertaken with prior approval from the Institute Animal Ethics Committee. (IAEC) Protocol number SVCOP/IAEC/020/2022-23. The room's temperature, relative humidity, and lighting were all maintained at a constant 24°C and a 12-hour light/dark cycle, respectively. The rats were housed in a typical cage. All test animals received standard pellet diet ad libitum and access to tap water.

Female albino rats were divided into five groups and each group consisted of six animals. The treatment was given as follows.

Group- I: Normal saline

Group- II: Control (Progesterone)

Group- III: Orlistat 20 mg/kg + Progesterone

Group- IV: Ethanolic extract of *Justicia tranquebariensis* (200mg/kg) + Progesterone

Group- V: Ethanolic extract of *Justicia tranquebariensis* (400 mg/kg) + Progesterone

A dose of 10 mg/kg bw of progesterone was subcutaneously injected into a rat's dorsal neck region for 28 days after being dissolved in arachis oil. Each drug has a dosage of 0.4 ml per 100 g of body weight. The test drugs were delivered 30 minutes before to progesterone administration. Throughout the study, each Rat's anthropometric measurements were tracked. According to accepted procedures, each animal's body weight was recorded. Body Weight was recorded every week using a weighing scale. Abdominal Circumference and Body Mass Index (BMI) was calculated. Several biochemical markers, including glucose, lipid profile (cholesterol, TG, HDL, LDL, and VLDL), were examined in the plasma samples [10].

Results and Discussion

Percentage yield of various extracts of *Justicia tranquebariensis* was calculated and tabulated in table 1.

Table 1. Percentage yield of different extracts of *Justicia tranquebariensis*

S.No	Extracts	(%Yield)
1	Ethanol	4.95%w/v
2	Chloroform	2.41%w/v
3	Pet ether	3.03%w/v

Preliminary phytochemical screening studies were carried out for various extracts of *Justicia tranquebariensis* and results are tabulated in Table 2.

Table 2. Preliminary phytochemical characterization of various extracts of *Justicia tranquebariensis*

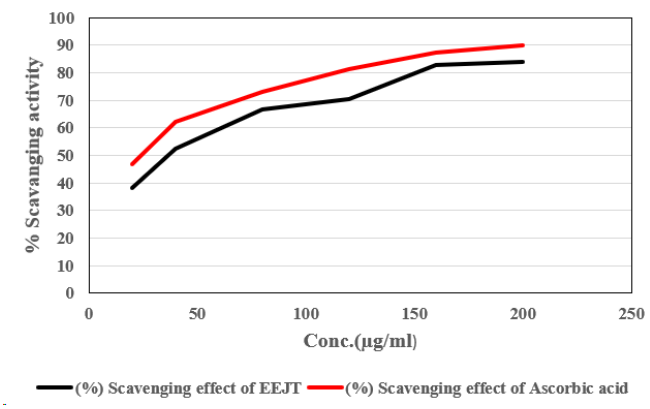
S.no	Phytochemical constituents	Hexane	Ethyl acetate	Chloroform	Pet ether	Ethanol	Distilled water
1	**Alkaloids**	+	+	++	+	+++	+
2	**Tannins and phenols**	_	_	+	_	+++	+
3	**Coumarin**	+	_	_	_	_	_
4	**Steroids**	_	+	+		++	+
5	**Saponins**	_	_	+	_	+	_
6	**Glycosides**	_	+	+	_	++	+
7	**Flavonoids**	+	+	+	_	++	+
8	**Terpenoids**	_	+	_	_	_	_

Total phenolic content (TPC) and Total Flavonoid content (TFC) of *Ethanolic extract of Justicia tranquebariensis* (EEJT)

In this study, *Justicia tranquebariensis* recorded to possess highest phenolic and flavonoid content in the ethanolic extract with 45.9±1.21 mg GAE/g of extract, 145.7 ±1.30 mg quercetin/100 mg of extract, respectively. TPC was calculated using the standard curve of gallic acid (standard equation curve equation: Y=0.0033x+0.0378, R2 =0.9761), TFC was calculated using the standard curve of quercetin (standard equation curve equation: Y=0.03576x + 0.211, R2 =0.9922).

In vitro Antioxidant Activity

In vitro antioxidant activity was carried out as the *ethanolic extract of Justicia tranquebariensis* (EEJT) contained high levels of polyphenol and flavonoid compounds. In addition, previous studies have also concluded that polyphenols and flavonoids are an important group of secondary metabolites with high biological activity, especially the ability to scavenge free radicals. Therefore, the study was conducted to investigate the in vitro antioxidant activity of *Justicia tranquebariensis* extract by 3 methods of DPPH, Nitric oxide and Hydroxyl radical scavenging activity. The study results are presented in the figures 1(a-c), which proved that antioxidant activity of *ethanolic extract of Justicia tranquebariensis* was increased in a concentration dependent manner. The study showed that EEJT exhibited antioxidant capacity on the three test methods with IC50 values of 38.18% (DPPH), 59.56% (NO) and 26.93% (H_2O_2) respectively when compared to standard Ascorbic acid.



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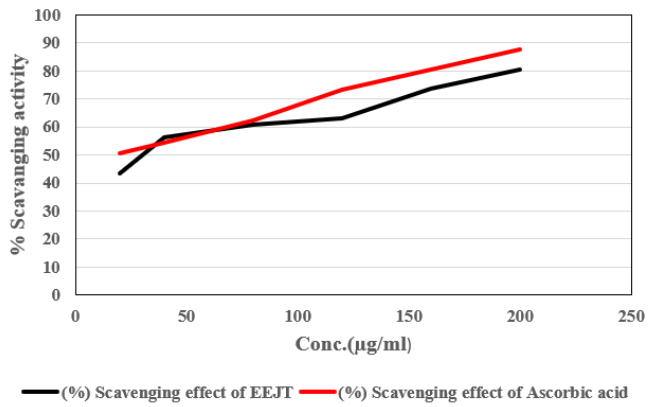

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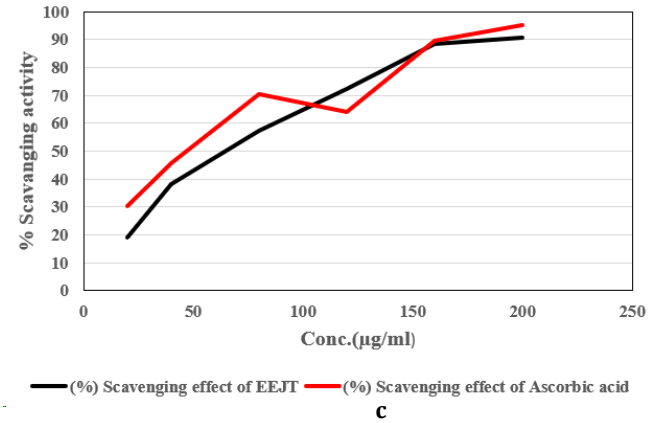

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Figure 1. In vitro Antioxidant Activity of ethanolic extract of *Justicia tranquebariensis*

a. DPPH Radical scavenging activity; b. Hydroxyl Radical Scavenging assay c. Nitric oxide radical scavenging assay

Anti-Obesity Activity of ethanolic extract of *Justicia tranquebariensis*

There were no significant alterations recorded in body weight of all animals in all groups till 7th day of study. The significant increase in body weight of animals were recorded in group-II ($p<0.05$) on 14th day and ($p<0.001$) on 21st and 28th day when compared to normal control group- I. Initially there were no significant difference was recorded in abdominal circumference of all animals in all groups. At the end of study, there was significant ($p<0.001$) increase in abdominal circumference recorded in group-II when compared to normal control. The increased abdominal circumference was found significantly ($p<0.001$) reduced in group-II, IV and V in equipotent manner when compared to group-II on 28th day. Group-II exhibited significant increase ($p< 0.001$) in BMI on the 28th day of study, when compared with the normal control group. At the end of study, the BMI was found to be significantly increased ($p<0.05$) in EEJT 400 mg/kg p.o. and standard group at 20 mg/kg. The BMI of EEJT 200 mg/kg p.o. was not significantly increased ($p>0.05$), when compared with the progesterone induction control group (Table 3).

Table 3. Effect of Ethanolic extract of *Justicia tranquebariensis* on progesterone induced alterations in physical parameters.

Groups	Bodyweight(gm)					Abdominal circumference (cm)		BMI (gm/cm²)	
	0th Day	7th day	14th day	21st day	28th day	Start	End	Start	End
Normal control	21 ±0.681	21.467 ±0.679	22.158 ±0.682	22.550 ±0.716	23.7 ±0.705	6.127 ±0.026	7.157 ±0.0442	500.128 ±2.720	509.588 ±2.99
Disease control	21 ±0.581ns	21.548 ±0.569ns	25.588 ±0.786###	28.25 ±0.8721###	31.92 ±1.234 ###	6.15 ±0.0244ns	7.43 ±0.1447 ###	533.5795 ±5.303##	593.14±4.927###
Standard	23.26 ±0.74ns	23.27 ±0.689ns	24.5 ±0.695ns	24.86 ±0.6427*	23.13 ±0.4671***	5.77 ±0.024ns	6.25 ±0.0478***	563.079 ±4.527***	569.253±6.954*
EEJT 200 mg/kg p.o.	23.5 ±0.97ns	23.56 ±0.659ns	26.5 ±0.672ns	26.1367 ±0.5141*	25.1 ±0.8***	5.88 ±0.011ns	6.25 ±0.0862***	564.0059 ±8.323**	575.104 ±5.19ns
EEJT 400 mg/kg p.o.	22.4 ±0.89ns	24.4 ±0.827ns	26.1 ±0.752ns	25.11 ±0.7354*	24.113 ±0.8417 ***	6.15 ±0.0326ns	6.327 ±0.0156***	558.3132 ±5.441*	561.28 ±5.26*

Values are the mean ± S.E.M. of six mice /treatment. nsp>0.05, #p<0.05, ##p<0.01, ###p<0.001 when compared to normal control group. Significance nsp>0.05, *p<0.05, **p<0.01, ***p<0.001, compared vs. progesterone control.

Effect of Ethanolic extract of *Justicia tranquebariensis*on progesterone induced alterations in lipid profile is tabulated in table 4. The parameters i.e., total cholesterol, triglyceride, LDL- cholesterol, VLDL, Atherogenic index were significantly ($p< 0.001$) increased in group-II as compared to normal control group-I. All these lipid profile parameters were significantly decreased ($p< 0.001$) in equipotent manner in group-III, IV and V when compared to group-II The level of HDL- cholesterol was significantly ($p< 0.001$) decreased in progesterone treated group-II as compared to normal control group and its level was significantly ($p< 0.001$) and equipotently restored in reference standard Orlistat treated group-III and Ethanolic extract of *Justicia tranquebariensis*treated group-IV & V when compared to progesterone treated group-II.

Table 4. Effect of Ethanolic extract of *Justicia tranquebariensis* on progesterone induced alterations in lipid profile
Values are the mean ± S.E.M. of six mice /treatment. nsP>0.05, #p<0.05, ##p<0.01, ###p<0.001 when compared to normal control group. Significance[ns]P>0.05,P<0.05, **P<0.01,***P<0.001, compared Vs. progesterone control.

Groups/ treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL (mg/dl)	VLDL (mg/kg)	HDL (mg/dl)	Atherogenic index
Normal control	114.82 ±1.74	83.178 ±0.8724	38.74±1.308	16.45±0.1445	27 ± 4.5875	0.475±0.021
Disease control	156.23 ± 1.856###	151.93± 1.014###	107 ± 2.280###	30.169 ± 0.2128###	13.669 ± 1.125###	1.08 ± 1.082###
Standard	121.823 ± 1.014***	106.678 ±1.944***	62.043 ± 2.538***	22.143±0.3887***	23.6± 1.627***	0.657 ± 0.038***
EEJT (200mg/kg) p.o.	125 ±2.572***	98 ±2.221***	57.5 ± 1.324***	19.7 ± 0.2322***	21 ± 7.5574***	0.67 ± 7.052***
EEJT (400 mg/kg) p.o.	121± 2.055***	91.67 ± 2.285***	50 ± 3.198***	18.34 ± 0.4651***	23.34 ± 0.4316***	0.595 ± 0.025***

Effect of Ethanolic extract of *Justicia tranquebariensis* on progesterone induced alterations in glucose level is tabulated in table 5. The glucose level was found significantly increased (p< 0.001) in group-II as compared to group-I whereas its level was significantly decreased (p< 0.001) in group-III and group-IV & V in equipotent manner when compared to group-II.

Table 5. Effect of Ethanolic extract of *Justicia tranquebariensis* on progesterone induced alterations in glucose level

S. No.	Group	Glucose level (mg/dl)
1	Normal control	75±2.943
2	Disease control	132.5±3.349###
3	Standard	85.332±2.455***
4	EEJT (200mg/kg)p.o.	84.338±1.620***
5	EEJT (400mg/kg)p.o.	79.667±1.547***

Values are the mean ± S.E.M. of six mice /treatment. ns P>0.05, #p<0.05, ##p<0.01, ###p<0.001 when compared to normal control group. Significance[s]P>0.05,*P<0.05, **P<0.01,***P<0.001, compared Vs. progesterone control.

Conclusion

The results revealed that the ethanolic extract of *Justicia tranquebariensis* has powerful antilipase potential compared with the positive control (Orlistat) and the extract has powerful activity on blood glucose levels. Moreover, the presence of flavonoids and phenols might be responsible for the antioxidant and anti obesity activity of the plant under study. The present work attempts to expand the applications of herbal medicine in the drug discovery system for the manufacturing of medicines from nature.

Conflicts of interest

The authors declare no conflicts of interest.

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Author Contribution

All authors are contributed equally.

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