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Evaluation of estrogenic potential of ethanolic and aqueous extract of Pitunia hybrida

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Abstract

The objective of the present study was to assess the estrogenic potential of different leaf extract of *Petunia hybrida* using the animal model. The extraction ability of different solvents for recovering extractable components from leaves followed the order: ethanol>chloroform>aqueous>pet ether. The findings of preliminary phytochemical analysis suggest the presence of alkaloids, saponin glycosides, phenolics, terpenoids, sterols, and flavonoids in the leaf of the plant. The total phenolic content of pet ether, chloroform, ethanol and aqueous extracts of *Petunia hybrida* were 16.18±0.89, 23.97±0.61, 71.77±0.59 and 31.38±0.36 GAE mg/g, respectively. All the extracts were subjected to *in vitro* determination of antioxidant potential by DPPH method. The IC_{50} value of the DPPH scavenging potential for the chlorofom, ethanol and aqueous extracts was found to be 245.9, 115.2 and 181.3µg/mL respectively whereas the IC_{50} value of the pet ether extract was more than 250µg/mL. The ethanolic and aqueous extracts exhibited higher antioxidant potential as well as phenolic content and were evaluated for estrogenic potential in rats by uterine weight assay method. It was found that the extracts exhibited significant estrogenic activity but not comparable to the standard drug ethinyl estradiol. The results obtained led to the conclusion that *Petunia hybrida* leaves are a rich source of potential antioxidants and also possess the capacity to increase the estrogen level in animal. Estrogen receptor binding assays may be further carried out using the extracts to confirm the estrogenic mechanism of *Petunia hybrida* extracts.

Keywords: *Petunia hybrida*, estrogenic, antioxidant, phenolic content, anthocyanin, DPPH

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Introduction

Hormones are the chemical messengers that are secreted by small organs called as glands into the blood stream and are transported by the blood to various organs or tissues to exert their physiological action.[1] Estrogen is thought to be the primary female hormone and it builds up the lining of the uterus, the breast tissue and leads to thickening of the vaginal walls. Progesterone is produced during the second half of the menstruation cycle and is known to prepare the lining of the uterus for egg implant and fertilization. Testosterone is the hormone responsible for sexual desires, generating vigor and energy.[2] Literature reveals the utilization of several plant extracts in modulating the estrogenic activity in experimental animals.[3-11] The presence of anthocyanin flavonoids was in most of these extracts was instrumental in the estrogenic action.

Petunia hybrida is a hybrid of various species of petunia produced by hybridization *P. axillaris* and *P. integrifolia. Petunia* is herbaceous perennial that is grown as an annual flowering plant throughout the summer. The flower of the plant is funnel shaped and the plant is bushier producing a number of small flowers around 5-7 cm in diameter (Figure 1).[12] The leaves of the plant are soft, hairy and smooth edged while the flowers are multicolored and may be striped, velvety and intense colored. The plant is known to contain some acylated anthocyanins[13] which are cinnamic acid, coumaryl rutinoside glycosides and rutinoside glucosides acylated with caffeic acid. Owing to the presence of several anthocyanin flavonoids, *Petunia hybrida* could be explored a number of pharmacological actions. Hence it was enivsioned to explore the estrogenic activity of *Petunia hybrida* leaf extracts.

Figure 1 *Petunia hybrida* flowering plant

Material and Methods

Preparation of the plant material

The leaves and flowers of *P. hybrida* were procured from Shubham Nursery, Bhopal in the month of January and authenticated at Saifia Science College, Bhopal. The voucher specimen was deposited in Department of Pharmacognosy, Technocrats Institute of Technology-Pharmacy, Bhopal, Madhya Pradesh for future reference. The leaves of the authenticated plant were washed with distilled water and were dried under shade. The dried leaves were powdered using a hand blender at low speed. The powdered leaves were stored in air tight container until they were required for extraction purpose.

Extraction of leaves and qualitative phytochemical screening[14,15]

The powdered leaves were used for the extraction process. Briefly 500 g of the leaf powder was packed

evenly in the extractor of the soxhlet apparatus and the phytoconstituents were extracted with various solvents of increasing polarity including petroleum ether, chloroform and ethanol (90%) by hot continuous extraction process for about 18 h. The aqueous extraction was carried out by cold maceration process after completion of the hot solvent extraction process. The extracts were filtered while hot through Whatman filter paper to remove any impurity. The extracts were concentrated by distillation to reduce the volume by 10 parts. The concentrated extracts were transferred to 100 ml beaker and the remaining solvents were evaporated on water bath. The oleo-resinous extracts were collected and placed in desiccators to remove the excessive moisture**.** The dried extracts were stored in desiccators for further processing.

The qualitative phytochemical screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids.

Total Phenolic content[16]

The extract was diluted to 100 mL with methanol and was stored at 4°C in amber bottles and served as the stock solution (50 mg/mL). For total phenolic content determination, 200 µL of sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After 3 min, 300 µL of 20% Na_2CO_3 aqueous solution was added and the mixture allowed standing for 2 h. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the analytical curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

DPPH radical scavenging assay[16]

Separately, 1mM solution of DPPH and extract solution (50-250 µg/mL) were prepared in ethanol. 1.5ml of the extract solution was added to 1.5 ml of DPPH solution. The absorbance was measured at 517 nm against the corresponding blank solution which was prepared using 3 mL ethanol. The control sample used was 3 mL of DPPH. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on control reading by following equation.

$$\text{DPPH scavenged (\%)} = \frac{(A_{con} - A_{test})}{A_{con}} \times 100$$

A_{con} - is the absorbance of the control reaction

A_{test} - is the absorbance in the presence of the sample of the extracts.

Determination of Estrogenic Activity

The *in vivo* estrogenic action of the extracts was evaluated in 25-30 days old female albino rats weighing between 150–180 g using modified uterine

weight method reported by Rubin et al[25]. The protocol of the present work was approved by Institutional Animal Ethical Committee (IAEC), Technocrats Institute of Technology-Pharmacy Bhopal, India (TIT/IAEC/831/P'Col/2020/03).

The animal were grouped and housed in poly acrylic cages (38x23x10 cm) in the animal house of the institute. Not more than four animals per cage were housed and maintained under standard laboratory conditions with natural dark and light cycle (14 h light/10 h dark) at 27±2°C and relative humidity (RH) 44-56% with free access to standard diet (Golden Feeds, India) and tap water *ad libitum* for one week for acclimatization before and during the experiments.

Animal were divided into four groups of 6 animals each for conducting the study.

Group I was administered 1% Tween 80, p.o. daily and served as the control

Group II was administered with ethinyl estradiol (standard) at 0.02 mg/kg body weight

Group III was administered with 50 mg/kg p.o. of the ethanolic extract

Group IV was administered with 50 mg/kg p.o. of the aqueous extract

The extract & standard drug were administered orally for first three days. On the fourth day (24h after the last dose administered) the animals were sacrificed by decapitation, uteri were dissected out & the surrounding tissues were removed (Ovariectomy). The weight of the uterus was recorded for each group of animals and the uterine ratio was calculated by dividing the uterine weight (mg) by the body weight of the animals (g) multiplied by 100.

Statistical Analysis

The results of pharmacological studies were expressed as mean ± SEM. The total variations present in data were evaluated by using Graph Pad Prism 5 project software one way ANOVA (analysis of variance) followed by Dunnett's multiple comparison Test. The result were considered statistically significant when P- value less than 0.001 ($P<0.001$) *vs* control.

Results and Discussion

The extraction abilitiy of different solvents for dissolving and extracting various components from leaves was in the order: ethanol > chloroform > water > pet ether.

The findings of the phytochemical analysis suggest the presence of alkaloids, saponin glycosides, phenolics, terpenoids, sterols, and flavonoids in the leaves. The presence of anthocyanins has been reported by Griesbach et al.[13] The total phenolic content of pet ether, chloroform, ethanol and aqueous extract of *Petunia hybrida* were 16.18±0.89, 23.97±0.61, 71.77±0.59 and 31.38±0.36 GAE mg/g, respectively. The ethanolic extracts exhibited highest amount of total polyphenol content compared to all other solvent extracts.

DPPH radicals scavenging activity

DPPH is stable nitrogen centered free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents, then losing colour stoichometrically with the number of electrons consumed, which is measured spectrophotometricallty at 517 nm. The results obtained (Figure 2) make it evident that that the extracts had the ability to donate hydrogen thereby stabilizing DPPH. The scavenging was found to dose dependent.

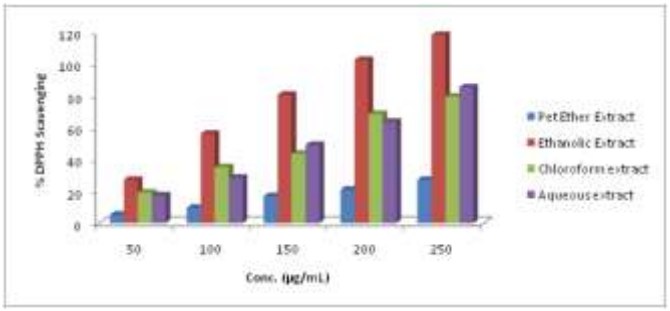

Figure 2 % DPPH scavenging potential of various extracts of *Petunia hybrida*

Determination of Estrogenic activity

The ethanolic and aqueous extracts were found to contain the higher phenolic content and antioxidant action and hence it was subjected to *in vivo* evaluation of their estrogenic activity as a function of the uterine weight in ovariectomized rats.

The uterine enlargement assay yielded significant differences ($p < 0.001$) in uterine weights among the treatment groups (Table 1). The estradiol, 50 mg/kg and 100 mg/kg ethanolic extract treated groups increased the uterine weight by 60, 124 and 197% respectively, compared to the control group. The results obtained reveal that both ethanolic and aqueous extracts of *Petunia hybrida* possess estrogenic potential but as thought far less than that of ethinyl estradiol. The estrogenic potencies for the standard drug, ethanolic and aqueous extracts was found to be 100000, 3 and 0.56 respectively, calculated as the dose required to increase 10 mg weight of the uterus.

Table 1 Estrogenic activity of extracts in ovariectomized rats

Group	Uterine Weight (mg ± SEM)
Control	17.77 ± 0.1453
Standard	28.45 ± 0.1784
Ethanolic extract (50 mg/kg)	39.85 ± 0.5685
Aqueous extract (50 mg/kg)	20.25 ± 0.3922

Values are mean ± SEM of six determinations

Conclusion

The objective of the present study was to assess the estrogenic potential of different leaf extract of *Petunia hybrida* using the animal model. The results obtained led to the conclusion that *Petunia hybrida* leaves are a rich source of potential antioxidants and also possess the capacity to increase the estrogen level in animal. Estrogen receptor binding assays may be further carried out using the extracts to confirm the estrogenic mechanism of *Petunia hybrida* extracts.

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