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Efficacy of different leaf extract fractions of *Prunus cerasoides* D. Don on benign prostate hyperplasia

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Abstract
Benign prostatic hyperplasia (BPH) is a common problem of aging for men. The most serious cases of BPH may be treated surgically. In addition to allopathy, traditional herbs were used as drugs against prostate diseases in ancient and folkfore systems. Various formulations of *P.cerasoides* listed in ancient and folkfore literature. Several Preliminary studies are carried out for its use as in prostate and genitourinary disorders, which require a systemic study and evaluation of for its use. Here, an attempt has been made to evaluate the beneficial effects of *P.cerasoides* on prostate and urinary disorders. Leaves of *P.cerasoides* were collected from Shillong, Meghalaya.
The methanolic extract of the plant was fractionated by column chromatography. For *in-vivo* studies, wistar male rats were divided into groups, first group received propylene glycol (0.2 ml/rat i.p.), second group received testosterone (0.2 mg/rat s.c.), third group received testosterone with plant extract (10 mg/kg body weight, i.p.) and the last group received testosterone with standard drug (Finasteride). One group of uncastrated rat received propylene glycol (0.2ml/rat i.p.). After 30 days all the animals were sacrificed under anesthesia and the total prostate weight was measured for all the animals. Plant extract significantly decreased the testosterone induced prostate weight.

Keywords: *Prunus cerasoides*, prostate hyperplasia

1. Introduction
Prostatic problem is commonly seen for men under 50, the most common prostate problem is prostatitis. From 40 years of age the prostate increases in volume by 2.4cm3 per year on average. For men, over 50 the most common prostate problem is prostate enlargement. This condition is called as Benign Prostatic Hyperplasia (BPH) [1]. Older men are at risk for prostate cancer as well. Affecting not just elderly men, prostate disorders are much more common than would be expected in middle-aged individuals. Over half of 40-59 year-old men have enlarged prostates, and, although most will not develop clinically significant disease, one fourth of 50-year olds have some cancerous cells in their prostate [2].

Prostate Gland
The prostate is part of a man's sex organs. It's about the size of a walnut and surrounds the tube called the urethra, located just below the bladder. It's a fibro musculo- glandular organ. It is situated in lesser pelvis 2cm behind the symphysis pubis and upper part of the pubic arch, above the superior part of the uro-genital diaphragm and in front of the ampulla of rectum. It's roughly 3cm in length, 4cm in breadth, 2cm in thickness. It weighs about 20gm [3].

Current Understanding of Prostate Growth
Testosterone, the main circulating androgen, is not the primary nutrient for the prostate. That role belongs to DHT, which is derived from testosterone within prostate cells by the action of the enzyme 5AR.11-13 Testosterone in serum has approximately 10 times the concentration of DHT, but in the prostate gland, the ratio is more or less reversed [4]. The biologic role of DHT was clarified by the work of Jean Wilson and coworkers at the University of Texas [5].

Prostate Disorders
The prostate is a small, walnut-sized gland found only in males located just below the bladder and surrounding the urethra, the tube through which urine flows during expulsion from the body. As males reach middle age, the prostate tends to grow larger, a natural and common occurrence [6]. As it grows, the prostate tends to constrict the urethra, making urination more difficult.
The prostate gland that surrounds the urethra that drains the bladder, prostate disorders often affect urination. The three most common disorders are 1) an inflammatory infection called prostatitis; 2) benign prostatic hyperplasia (BPH), a prevalent non-cancerous enlargement of the prostate; and 3) cancer, the most frequent male malignancy [7].

Benign prostatic hyperplasia (BPH)
Benign prostate hypertrophy (BPH) is a non-cancerous swelling of the prostate gland that may interfere with the flow of urine from the bladder in men [8].The condition is also known as benign prostatic hypertrophy and, more accurately, as benign prostate hyperplasia (the proliferation of cells in the prostate gland). BPH is a very common condition among men; nearly half of all men aged 50 have the condition, and by age 80, the percentage of men with BPH climbs to 75 percent. Benign prostate hypertrophy is treated by allopathic (conventional Western) physicians by changes in lifestyle, medications, and/or surgery. Practitioners of Traditional Chinese Medicine (TCM) rely on methods that avoid the side effects of medication and surgery, such as acupuncture or herbal remedies, for treatment of the disorder [9].
Two classes of drugs serve as the initial treatment of choice for most men with benign prostatic hyperplasia (BPH): 5_-reductase inhibitors and _-adrenergic receptor blocking agents.

The natural prostrate remedy is said to be the best treatment option among all the natural remedies for prostrate disorders. Of late, some doctors in the U.S. have also begun to use this natural remedy in treating their patients [10].Tripathy (2004) has carried out a studies of leaf extract of *P.cerasoides* on prostate and urinary disorder and he found significant effect on lowering the prostate weight [11].Present study is carried out to see the capability of *P.cerasoides* leaf extract fractions to reduce the testosterone induced prostate weight of rat [12].

The Plant *Prunus Cerasoides D.Don*

Prunus cerasoides (Family Rosaceae) the Himalayan Wild Cherry is a sacred plant in Hindu mythology. It is found in Sikkim, Nepal, Bhutan, Myanmar, West China and India [13].
The tree thrives in well-drained and moisture-retentive loamy soil. It will grow well with a bit of lime in the soil, but is likely to become chlorotic if too much lime is present [14]. It requires an open, sunny and sheltered location.

2. Materials and methods

2.1 Collection of plant material

Leaves of P.cerasoides were collected from Shillong, Meghalaya with help of some local khasi peoples.The Taxonomical identification was done by Botanical Survey of India, Shillong (Meghalaya).The collected leaves of P.cerasoides were then air dried, powdered and stored in an air tight container for future use [15].

2.2 Pharmacognostic studies

2.2.1 Macroscopic/ organoleptic evaluation of the drugs:

Organoleptic (i.e. impression on the organs) refers to evaluation by means of organs of sense and includes the macroscopic appearance, odor, taste etc. of the drugs.

2.2.2 Determination of ash value

(A) Total Ash, Method

3 gm. Of the grounded air–dried drug was accurately weighed in a silica crucible and was incinerated at a temperature 700^0C to free from carbon. It was then cooled in dessicator and weighed, again incinerated at 700^0C and cooled in dessicator until attained a constant weight. Ash again treated with ammonium carbonate solution, dried and kept in dessicator. The percentage of total ash with reference to the air–dried drug was calculated [16].

(b) Acid insoluble ash, Method

The total ash was boiled with 25 ml of 2 M hydrochloric acid for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited, cooled in a desiccators and weighed. The percentage of acid–insoluble ash with reference to the air–dried drug was calculated.

(c) Water soluble ash, **Method**

The total ash was boiled with 25 ml of water for five minutes and the insoluble matter was collected on an ash less filter paper, washed with the hot water, and ignited for 15 minutes at a temperature not exceeding 450^0C. By subtracting the weight of insoluble part from that of total ash, the weight of the soluble part of ash was obtained.

2.2.3 Determination of extractive value

(a) Alcohol Soluble Extractive

5 g. of the coarsely powdered air–dried drug was macerated with 100 ml of alcohol in a closed flask for 24 hours, frequently shaked during the first 6 hours and was allowed to stand for 18 hours. There after it was filtered rapidly. 25 ml of the filtrate was evaporated to dryness in a tared flat bottom shallow dish, dried at 105^0C and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air dried drug.

(b) Water Soluble Extractive

5 gm. of coarsely powdered air dried drug was macerated with 100 ml. of water in a closed flask for 24 hours, frequently shaked during first 6 hours and was allowed t stand for18 hours. Thereafter it was filtered rapidly, 25 ml. of this filtrate was evaporated to dryness in a tared flat bottom shallow dish, dried at 105^0C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug.

Extraction

500gm of powdered leaves of P.cerasoides were extracted with 2.5 ltr of methanol in a Soxhlet apparatus. The solvent was then removed under reduced pressure. A greenish-brown colored semisolid weighing 5gm was stored. The extract was then washed with various solvent systems according to their polarity, viz., petroleum ether, chloroform and carbon tetrachloride

Animals

Healthy adult wistar albino rats weighing 50-80 gm. were procured. The animals maintained in standard laboratory conditions of temperature, humidity and 12 hours light and dark cycles. The animals were fed with standard feed and water. The place where the experiments were conducted was kept very hygienic by cleaning with antiseptic solutions. The husk for the purpose of keeping as a bed to the animals was cleaned daily before the experiment.

Surgical procedures

After one week of acclimatization, castration of 15 rats were carried out with ketamine anaesthesia and strict senitized condition [17].Castration was performed via the scrotal route by removing epididymal fatty pads with testicles. Operated animals were sutured, and the injured areas were disinfected with povidine-iodine ointment. The operated animals were treated with antibiotic to prevent infection. The animals were rested for another 15 days.

Treatments

Now 8 animals were divided into 4 groups. First group containing 2 normal rats receiving vehicle (Propylene glycol), second group containing 2 castrated rats receiving vehicle (Propylene glycol), third group containing 2 castrated rats receiving testosterone and fourth group containing 2 castrated rats receiving testosterone(0.2 mg/rat s.c.) and plant extract (10 mg/kg i.p.). All the treatments were carried out for 30 days.

In pharmacological test the doses used are usually based on human doses per kg of rat [18]. The dose of *P.cerasoides* was based on the studies by Caballero *et al*. 2003. All the treatments were carried out in alternate days for a period of 30 days. The testosterone (Aquaviron) was administered subcutaneously in the cervical back region with a total injection volume of 0.2 ml. The first two groups of normal and castrated received the same volume of vehicle (Propylene glycol). Dried plant extract fractions were dissolved in propylene glycol and administered i.p. with total injection volume of 0.2 ml.

The nocturnal urine volume was measured at an interval of 3 days. All the animals were weighed at the beginning of the experiments and then once a week during the experiment.

At the end of 30 days all the animals were sacrificed under anaesthesia and prostate were removed from all the treated animals for its weight.

3. Result and discussion

The leaf of *Prunus Cerasoides* D. Don. (Rosaceae) has been investigated in a systematic way covering pharmacognostical and pharmacological aspects to rationalize its use as a drug of therapeutic importance in case of prostate and urinary disorder.

3.1 Effect of the different fractions of leaf extract of *P. cerasoides*

After determining the chromatographic profile of the crude methanolic extract of *P. cerasoides*, three fractions (Fraction I, Fraction II and Fraction III) are taken into consideration for determining its biological activity in prostate disorder on rat. The prostate weight of the testosterone induced castrated rats after being treated with the fractions are presented in Table and Figure.

The Fraction III treated group shows the lessened effect of testosterone on the prostate gland enlargement comparing to that of group I and group II treated group.

3.2 Testosterone effect on the maintenance of the prostate size:

The pair wise statistical analysis carried out for prostate gland weight in the group I (normal control) and group II (castrated control) suggested that there was a significant difference in the mean weight (112±8) and (65±15) mg respectively. It has seen that the size of the prostate decreases significantly in comparison with normal control.

On comparing group II (castrated control) with group III (Testosterone treated), a highly significant increase in the prostate size noted in case of group III animals with mean prostate weight (390±25) mg.

There was a highly significant increase in mean prostate weight value of the group III when compared with that of group I (normal control).

On comparing group IV and group V (castrated standard drug treated with testosterone) no significant difference in the mean prostate weight.

3.3 Determination of Quantitative standards

Table 1: Quantitative fractions of *P. cerasoides*

S. No.		Parameters	Values of 3 Replicates (%)	Mean (%)
1.	Ash Value	a) Total ash	3.0%	3.23%
			3.7%	
			3.0%	
		b) Acid-insoluble ash	0	0
		c) water soluble ash	0	0
2.	Extractive Value	a) Water soluble extract	23.60%	22.22%
			22.46%	
			20.60%	
		b) Alcohol soluble extract	27.60%	27.53%
			26.28%	
			28.73%	
3.	Loss on drying		2.70%	2.73%
			2.60%	
			2.90%	

3.4 Ash values

The ash values (Total ash, acid-insoluble ash, water soluble ash) of powder leaf of *P.cerasoide*s is presented in the table-1.The determination of ash use for detecting low grade product, exhausted drugs and excess of sandy or earthy matter. The ash of any organic material composed of their nonvolatile organic components. Controlled incineration of crude drugs results in ash residue consisting of an inorganic material (metallic salts and silica). This parameter used for the determination of inorganic materials, such carbonate, silicates, oxalates, and phosphates. Heating causes the loss of organic material in the form of CO_2 leaving behind the inorganic components. We can detect the extent of adulteration as well as establish the quality and purity of the drug by this method. A total ash figure used to exclude drugs which have been coated with chalk, lime to improve the appearance. Here the value obtained for the leaf of *P.cerasoides* is around 3.23 percent as total ash. The acid insoluble ash determines the acid insoluble material present in the drug material often of more value than the total ash because of the solubility of calcium oxide, calcium oxalate yielded by incineration and the value for the leaf of *P.cerasoides* was found 0 which revealed the inorganic material left after incineration was completely soluble in hydrochloric acid and the water soluble ash determines the water soluble material specifically the water soluble inorganic salts. The value was found to be 0 which revealed the complete insolubility of inorganic salt in water [19].

3.5 Extractive value

The extractive values of leaf of *P.cerasoide*s is presented in the table-1.

The extraction of any crude drug with particular solvent yields a solution containing different phyto-constituents. The composition of these phyto-constituents in that particular solvent depends upon the nature of the drug and solvent used. The use of a single solvent can be the means of providing preliminary information on the quality of a particular drug sample. The extractive value of the crude drug determines the quality as well as purity of the drug material. The ethanol and water soluble extractive values of the leaf of P.cerasoides was found to be 22.22 % and 27.53 % respectively.

3.6 Loss on drying

Loss on drying values of leaf of *P.cerasoide*s is presented in the table-1.

The loss on drying of bark powder was found to be 2.73 %. It revealed a considerable amount of moisture in the bark materials. The percentage of active chemical constituents in crude drugs is mentioned on air-dried basis. Hence, the moisture content of a drug should be determined and also be controlled to make the solution of definite strength. The moisture content of a drug should be minimized in order to prevent decomposition of crude drug either due to chemical change or due to microbial contamination. The objective of drying of fresh material is to fix their constituents i.e. to check enzymatic or hydrolytic reactions that might alter the chemical composition of the drug and to reduce their weight and bulk. Not only the ultimate dryness of the crude drug is important, but the rate at which the moisture is removed and the conditions under which it is removed also. If the rate is too slow, much spoilage may occur before the drying process is completed. Therefore, in general, drying should be accomplished as rapidly as possible [20].

Chromatographic Profile of Crude Extract of *Prunus cerasoides* D. Don

Table-2

Extract	Solvent System	No. of Spots	RF Values
Methanol	Carbon tetrachloride: Ethyl acetate (5:1)	4	0.40
			0.65
			0.70
			0.84
Petroleum ether	Chloroform: Methanol (9:1)	1	0.70
Chloroform	n-Hexane: Ethyl acetate: Glacial acetic acid (5:1:0.5)	2	0.50
			0.75

Chromatographic Profile of Different Fractions of Methanolic Extract of *Prunus cerasoides* D. Don

Table-3

Fractions	Solvent System	No. of Spots	RF Value
Fraction I	Carbon tetrachloride: Ethyl acetate (5:1)	2	0.42
			0.22
Fraction II	Carbon tetrachloride: Ethyl acetate (5:2)	2	0.38
			0.65
Fraction III	Carbontetrachloride: Ethylacetate (5:2)	1	0.44
Fraction IV	Carbon tetrachloride: Ethyl acetate (5:2)	2	0.40,0.60

Fraction I, II and III treated groups.

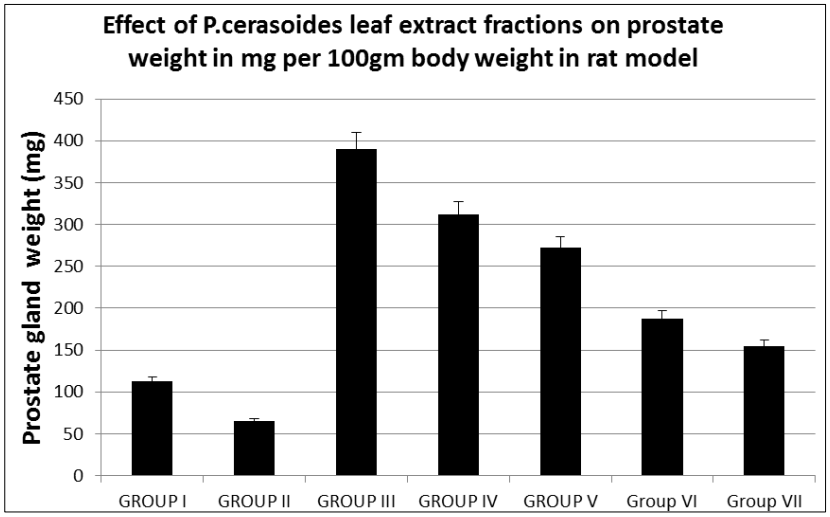

Fig 1

Table 4

Groups	Prostate weight in mg/100gm body weight
I	112±8
II	65±15
III	390±25
IV	311±20
V	272±18
VI	188±30
VII	154±25

- **Group I:** Normal uncastrated control (0.2ml propylene glycol/rat, i.p.)
- **Group II:** Castrated control (0.2 ml propylene glycol/rat,i.p.)
- **Group III:** Castrated rats receiving 0.2 mg testosterone/rat,s.c.
- **Group IV:** Castrated receiving 0.2mg testosterone s.c, and 10 mg/kg body weight fraction I of *P.cerasodes* leaf extract, i.p.
- **Group V:** Castrated receiving 0.2mg testosterone s.c, and 10 mg/kg body weight fraction II of *P.cerasodes* leaf extract, i.p.
- **Group VI:** Castrated receiving 0.2mg testosterone s.c, and 10 mg/kg body weight fraction III of *P.cerasodes* leaf extract, i.p.
- **Group VII:** Castrated receiving 0.2 mg testosterone,s.c. and 0.02 mg Finasteride i.p.

No significant difference between group VI and group VII (student t-test)

In Fraction I and Fraction II treated groups, the leaf extract fractions of *P.cerasoides* are not showing any significant effect in lowering the testosterone induced prostate weight. But in case of Fraction III treated groups, the leaf extract fractions shows a significant effect in lowering the prostate gland weight.

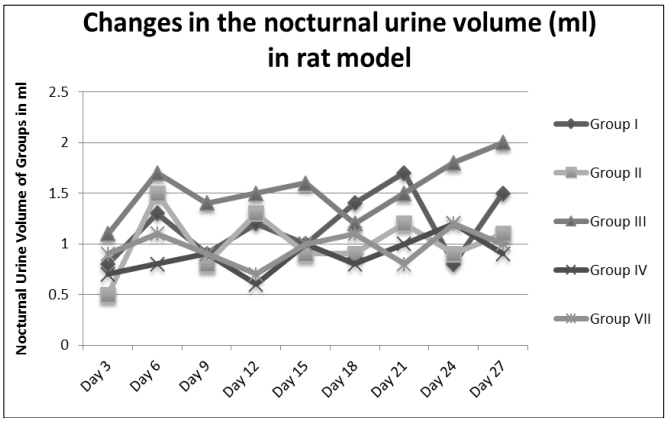

Fig 2

- **Group I:** Normal uncastrated control (0.2ml propylene glycol/rat, i.p.)
- **Group II:** Castrated control (0.2 ml propylene glycol/rat,i.p.)
- **Group III:** Castrated rats receiving 0.2 mg testosterone/rat,s.c.
- **Group IV:** Castrated receiving 0.2mg testosterone s.c, and 10 mg/kg body weight fraction I of *P.cerasodes* leaf extract, i.p.
- **Group V:** Castrated receiving 0.2mg testosterone s.c, and 10 mg/kg body weight fraction II of *P.cerasodes* leaf extract, i.p.
- **Group VI:** Castrated receiving 0.2mg testosterone s.c, and 10 mg/kg body weight fraction III of *P.cerasodes* leaf extract, i.p.
- **Group VII:** Castrated receiving 0.2 mg testosterone,s.c. and 0.02 mg Finasteride i.p.

Nocturnal urine volumes (ml) were collected at an interval of three days for the 3rd fraction treated groups. Data are presented in Figure-9.

The mean nocturnal urinary volume collected for all groups were apparently different. But no significant difference of urine volume in group VI and group VII.

4. Conclusion

Herbal medicine has been used in India for thousands of years and is increasingly been used worldwide during the last few decades as evidenced by rapidly growing global and national markets of herbal drugs [21]. According to WHO estimates, the present demand for medicinal plants is ~US $14 billion a year and by the year 2050 it would be ~US $5 trillion. Due to high prices and harmful side effects of synthetic drugs, people rely more on herbal drugs and this trend is growing, not only in developing countries but in developed countries too [22].

BPH appears to be stimulated by dihydrotestosterone (DHT). There are of course some similarities between the rat and human prostate. Various workers proved the proliferative effect of androgen on prostate [23]. Present study verified the effectivity of testosterone on the growth and maintenance of the rat prostate. The leaf extract of *P.cerasoides* showed the capability to reduce the testosterone induced prostate weight of rat.

Hence it may be concluded that the locally available plant *P.cerasoides* D.Don. (Rosaceae) showed the beneficial effect to reduce the testosterone induced prostate growth and can be recommended for further scientific investigation to develop as a drug.

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