

## Short Communication

# A comparative experimental study to evaluate *Mutral* (diuretic) activity of *Bilva Moola* and *Patra* (*Aegle marmelos* Corr.)

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Access this article online

Website: [www.ayujournal.org](http://www.ayujournal.org)

DOI: 10.4103/0974-8520.153790

Quick Response Code:



## Abstract

**Background:** *Bilva Moola* (root of *Aegle marmelos* Corr.) is one of the ingredients of *Dashamoola*. According to *Siddha Bhaishaja Manimala*, therapeutical properties denoted for the official part used are to be considered for all the other parts of the same plant. If the leaf of the plant is equally effective as the root then the destruction of the whole plant can be prevented. **Aim:** Study is planned to assess whether the leaves of *Bilva* can be substituted for its root by using the parameter of diuretic activity in experimental animals. **Materials and Methods:** Both samples *Bilva Moola* and *Patra* were administered to experimental rats by oral route at dose of 450 mg/kg. Control group received only normal saline. The diuretic effect was evaluated by measuring urine volume, sodium, potassium content in urine. Glucose, bilirubin, ketone, specific gravity, blood, pH of urine, protein, and urobilinogen, were also measured. **Results:** Both test drugs showed increase in urine output but root sample showed maximum output compared to leaves treated and control group of rats. Sodium and potassium level is found more pronounced in root than the leaf. There is a mild increase in urine pH for both the drugs. There is absence of glucose, bilirubin, ketone and haemoglobin in all the groups. **Conclusion:** The root sample of the plant showed a greater effect as diuretic in comparison to leaf, hence as per the concept leaves may replace root in *Dashamoola* and can be used as *Shothahara* but effect may be less compared to root part.

**Key words:** *Aegle marmelos*, *Bilva*, *Dashamoola*, diuretics, substitute

## Introduction

*Bilva Moola* (roots of *Aegle marmelos* Corr.) is one of the ingredients of *Dashamoola* and *Brihat Panchamoola*. It takes many years to grow a matured tree, to obtain root a plant has to be uprooted entirely, thus destroying the plant forever. In addition, Red Data List of Indian Plants puts *A. marmelos* in vulnerable threat status.<sup>[1]</sup> There is a greater need to discover suitable substitutes for it. *Bilva Moola* has *Mutral* (diuretic) activity<sup>[2]</sup> and is useful in *Ashmari* (urinary calculi), *Sharkara* (concretions), *Mutrakrichha* (dysurea),<sup>[3]</sup> *Mutraghata* (anuria), *Shotha* (oedema); while *Bilva Patra* (leaves of *Aegle marmelos*) is useful in *Shotha*.<sup>[4]</sup> *Dashamoola* is well known *Shothahara* *Dashamani* of *Charaka Samhita*. *Shothahara*

is often interpreted as anti-inflammatory activity. *Shothahara* *Dravyas* removes *Srotorodha* doing *Pachana* (digestion) and *Anulomana* of *Malabhaga Doshas* (metabolic waste). According to ancient Acharyas of Ayurveda *Mutra* (urine) is liquid *Malas* (wastes) or waste products which are formed and excreted after *Pachana* of the ingested food and the substances which promote urine formation are known as *Mutral Dravyas*. So, *Mutral* activity is one part of *Shothahara Karma*. According to *Siddha Bhaishaja Manimala*, therapeutical properties denoted for the official part used are to be considered for all the other parts of the same plant.<sup>[5]</sup> To stop destroying plants, it would be useful to evaluate diuretic activity of *Bilva Moola* and *Patra* experimentally to establish the efficacy of *Bilva Patra*. Pharmacological methods as per modern concepts provide a scientific base for therapeutic use of *Bilva Moola* and *Patra*. An experimental study was designed, to establish the leaf of *Bilva* (*A. marmelos* Corr.) as a substituted for its root and pharmacological evaluation of root and leaves part of *A. marmelos* Corr. (*Bilva*) for their diuretic effects in experimental animals.

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## Materials and Methods

### Procurement and preparation of test drug

Root and leaves of *Bilva* was collected from the Jamnagar area. The parts of *Bilva* were identified and authenticated by comparing it with different floras. The drug was thoroughly washed with water and then roots and leaves were shade dried. The parts of *Bilva* were pulverized to a fine powder (mesh no. number 80) and stored in airtight glass container for experimental purposes.

### Animals

Wistar strain albino rats of either sex and weighing between 180 and 220 g were obtained from the animal house attached to the Institute. The rats were maintained on Amrut brand animal pellet feed of Pranav Agro Industries, and drinking water was given *ad-libitum*. The animals were acclimatized 1 week before experimentation period. The animals were exposed to 12 h light and 12 h dark cycle with the relative humidity of 50–70% and the ambient temperature  $22 \pm 3^\circ\text{C}$  during the experimental period. All animals were kept in the same environmental conditions. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC/12/2012/05) in accordance with the guidelines formulated by CPCSE, India.

### Dose fixation and route of administration

The dose for experimental study was calculated by extrapolating the human dose to animal dose based on the body surface area ratio using the table of Paget and Barnes.<sup>[6]</sup> Therapeutic human dose  $\times$  surface area ratio (convertibility factor) for rats.

Human dose = 5000 mg/day<sup>[7]</sup>

Rat dose =  $5000 \times 0.018$  (conversion factor or body surface area for man to rat).

= 90 mg/200 g rat = 450 mg/kg body weight of rats.

### Preparation of test sample

Stock solutions of samples were prepared by dissolving 10 mg of dried sample in 10 ml of distilled water.

### Route of drug administration

The vehicle and test drug were administered by oral route with the help of a (feeding tube) gastric catheter sleeved to the disposable syringe.

### Grouping

Diuretic activity was evaluated in 18 Wistar strain of albino rats of either sex. The animals were divided into three groups (six animals in each)

- Group I: Control group
- Group II: Root powder of *A. marmelos* Corr.
- Group III: Leaves powder of *A. marmelos* Corr.

### Diuretic activity

Diuretic agent increases the quantity of urine. Normal urine output in rats is very small (1–2 ml/rat/day) hence to get the measurable quantity, the animals are to be hydrated. The urine output increases after administration of diuretics. Increases in volume of urine is measured and compared with normal urine output.

The diuretic activity was carried out as per procedure described by Gillard *et al.*<sup>[8]</sup> Rats were allowed free access to water and food till the start of the experiment. The overnight fasted rats were administered distilled water in the dose of 5 ml/100 g body weight to ensure uniform hydration. After oral drug administration, the rats were placed in individual metabolic cages with netted floor, and urine was collected for a period of 5 h in conical flasks placed below the polythene funnel of the metabolic cages. Collected volume of urine of each rat was noted down. A urine sample was taken for the estimation of sodium and potassium by flame photometry. Glucose, bilirubin, ketone, specific gravity, blood, pH of urine, protein, and urobilinogen, were also measured using standard Ames multiple strips.

### Statistical analysis

The obtained data has been presented as mean  $\pm$  standard error of the mean, difference between the groups, statistically determined by Student's *t*-test for unpaired data to assess the statistical significance between the groups.  $P < 0.05$  is considered as statistically significant.

## Results and Observations

Table 1 shows data of the urine volume for diuretic activity of the plant, in which both test drugs showed increase in urine output. The root sample showed maximum excretion of urine volume compared to leaves treated rats and control group of rats. Data of the Table 2 reveals that the root showed a maximum percentage excretion of sodium (45.44%) and potassium (47.98%) in urine compared to control group. *Bilva* leaves showed decrease in sodium excretion and increase in potassium excretion in urine compared to control group. The activity of root is more pronounced than the leaf drug. Table 3 shows the effect of test drugs on pH and specific gravity of urine in hydrated rats. The results showed a mild increase in urine pH for both the drugs. The test drugs did not affect the specific gravity of urine compared to control group. Table 4 shows the effect of test drugs on glucose, bilirubin, ketone and haemoglobin in urine of hydrated rats. The results show that all four parameters were absent in the urine of all rats. Table 5 shows the effect of test drugs on protein and urobilinogen of urine in hydrated rats. The result showed an increase in urine protein content for both parts of drug. However changes were statistically insignificant. That shows parts of the drug do not hamper kidney function. *Bilva* root showed decrease in urobilinogen excretion compared to control group. The activity of root is more pronounced than the leaf drug.

**Table 1: Effect of *A. marmelos* on urine volume in hydrated rats**

Groups	Volume of urine collected (ml/100 g/5 h)	Percentage change
Control	0.833 $\pm$ 0.160	-
<i>Bilva</i> (R)	1.312 $\pm$ 0.363	57.50 $\uparrow$
<i>Bilva</i> (L)	0.696 $\pm$ 0.325	16.44 $\uparrow$

Data: Mean $\pm$ SEM.  $\uparrow$ : Increase, *A. marmelos*: *Aegle marmelos*, SEM: Standard error of the mean, R: Roots, L: Leaves

**Table 2: Effect of *A. marmelos* on urinary excretion of Na<sup>+</sup> and K<sup>+</sup> in hydrated rats**

Groups	Sodium (mEq/L)	Percentage change	Potassium (mEq/L)	Percentage change
Control	5.352±3.567	-	3.903±2.078	-
<i>Bilva</i> (R)	7.784±3.969	45.44↑	5.180±1.915	32.71↑
<i>Bilva</i> (L)	2.784±0.932	47.98↓	5.724±1.815	46.65↑

Data: Mean±SEM. ↑: Increase, ↓: Decrease, SEM: Standard error of the mean, *A. marmelos*: *Aegle marmelos*, R: Roots, L: Leaves

**Table 3: Effect of *A. marmelos* on pH and specific gravity of urine of hydrated rats**

Groups	pH	Percentage change	Specific gravity	Percentage change
Control	7.083±0.154	-	1.013±0.00214	-
<i>Bilva</i> (R)	7.250±0.171	2.357↑	1.015±0.00342	0.197↑
<i>Bilva</i> (L)	7.633±0.203	7.765↑	1.011±0.00417	0.197↓

Data: Mean±SEM. ↑: Increase, ↓: Decrease, *A. marmelos*: *Aegle marmelos*, SEM: Standard error of the mean, R: Roots, L: Leaves

**Table 4: Effect of *A. marmelos* on glucose, bilirubin, ketone and haemoglobin in urine of hydrated rats**

Groups	Glucose (mg/dl)	Bilirubin (mg/dl)	Ketone (mg/dl)	Haemoglobin (mg/dl)
Control	Absent	Absent	Absent	Absent
<i>Bilva</i> (R)	Absent	Absent	Absent	Absent
<i>Bilva</i> (L)	Absent	Absent	Absent	Absent

*A. marmelos*: *Aegle marmelos*, R: Roots, L: Leaves

**Table 5: Effect of *A. marmelos* on protien and urobilinogen in urine of hydrated rats**

Groups	Protien (mg/dl)	Percentage change	Urobilinogen (mg/dl)	Percentage change
Control	0.667±0.333	-	1.000±0.000	-
<i>Bilva</i> (R)	1.167±0.401	74.96↑	0.833±0.167	16.7↓
<i>Bilva</i> (L)	1.000±0.632	49.92↑	1.000±0.000	0

Data: Mean±SEM. ↑: Increase, ↓: Decrease, SEM: Standard error of the mean, *A. marmelos*: *Aegle marmelos*, R: Roots, L: Leaves

## Discussion

*Bilva Moola* is one of the ingredients of *Dashamoola*. The aim was to find *Dashamoola* Substitutes. Findings of the conducted experiments have shown that *Bilva Patra* may be substituted for *Bilva Moola* in certain conditions. Diuretics are drugs that increase the rate of urine flow and excretion of electrolytes. The present study demonstrated the diuretic activity of root and leaves of *A. marmelos* Corr. In this study, root and leaves markedly enhanced urine volume and potassium level in urine. Root of *Bilva* showed marked increase in sodium level, while leaves decreased sodium level compared to control group. *Bilva* root has more pronounced activity than *Bilva* leaves [Table 6]. The observed pattern of diuretic is similar to the pattern observed with thiazide diuretics which increase the sodium and potassium and hence resulting in an increase of urine formation.<sup>[9-11]</sup> Diuretics act by decreasing the re-absorption of sodium in the convoluted

**Table 6: Consolidate statement showing the effects of *Bilva* leaves with *Bilva* roots on different urine parameters in rats**

Parameters	Groups	
	<i>Bilva</i> root	<i>Bilva</i> leaves
Urine volume	NSI	NSI
Na <sup>+</sup> excretion	NSI	NSD
K <sup>+</sup> excretion	NSI	NSI
pH	NSI	NSI
Specific gravity	NSE	NSE
Glucose	Absent	Absent
Bilirubin	Absent	Absent
Ketone	Absent	Absent
Haemoglobin	Absent	Absent
Protien	NSI	NSI
Urobilinogen	NSD	NSE

NSI: Non significant increase, NSD: Non significant decrease, NSE: Non significant effect

tubules. The root sample of the plant shows a greater effect as diuretic in comparison to leaves, hence as per the concept leaves may replace root in *Dashamoola* which is mainly indicated for *Shotha*. *Shotha* can be interpreted as edema, for this, one of the treatment is diuretics, but effect may be less compared to root part. Human physiology differs from animal physiology and animal studies have own limitation. The root sample of the plant shows a greater effect as diuretic in comparison to leaf, hence as per the concept leaves may replace root in *Dashamoola* and can be used as *Shothahara* but effect may be less compared to root part. Human physiology differs from animal physiology and animal studies have own limitations further clinical study may be carried out to prove the above said claims.

## Conclusion

Both root and leaves of *Bilva*, showed better diuretic activity in albino rats at dose level of 90 mg/ 200 g. However, *Bilva* root has more pronounced diuretic activity in comparison to *Bilva* leaves. As per the concept adapted, *Bilva* leaves may be substituted for *Bilva* root individually as well as in *Dashamoola* as diuretic. Keeping this in mind, it can be suggested to prefer *Bilva* leaves instead of *Bilva* root which facilitates in preventing destruction of whole plant.

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**How to cite this article:** Parmar NA, Patel BR, Nariya MB. A comparative experimental study to evaluate *Mutrala* (diuretic) activity of *Bilva Moola* and *Patra* (*Aegle marmelos* Corr.). *Ayu* 2014;35:344-7.

**Source of Support:** Nil, **Conflict of Interest:** None declared.

## हिन्दी सारांश

### बिल्वमूल एवं पत्र के बीच मूत्रलकर्म का तुलनात्मक परीक्षण

नेहा ए. परमार, भूपेश आर. पटेल, मुकेश बी. नारिया

बिल्वमूल दशमूल का एक घटक है। सिद्धभैषज्य मणिमाला के अनुसार वनस्पति के हर भाग में समान गुणधर्म कर्म रहते हैं। बिल्व का निकंदन रोकने को बिल्वपत्र की कर्मशक्ति जानने के लिए बिल्वमूल एवं पत्र के बीच मूत्रलकर्म का तुलनात्मक परीक्षण करना जरूरी है। द्रव्य के दोनों नमूने चूहों के दो वर्ग में मुखमार्ग से ४५० मि.ग्रा./कि.ग्रा. मात्रा में दिये गये। जबकि नियंत्रित वर्ग को सिर्फ साधारण जल दिया गया। मूत्रल कर्म का परीक्षण मूत्र मात्रा से तथा सोडियम, पोटेशियम के मूत्र में प्रमाण से किया गया। एवं ग्लूकोज, बिलिरुबिन, कीटोन, विशिष्ट गुरुत्व, रक्त, मूत्र की क्षारता, प्रोटीन तथा यूरोबिलिनोजन को भी प्रमाणित एम्स मल्टीपल स्ट्रीप्स से मापा गया। मूल का नमूना पत्र के नमूने से बेहतर मूत्रल साबित हुआ। इसलिए दशमूल में बिल्वमूल के अभाव में बिल्वपत्र का उपयोग कर सकते हैं। किन्तु द्रव्य की मूत्रल कर्म में कमी पाई जा सकती है।