# DIURETIC ACTIVITY OF AQUEOUS EXTRACTS OF *MICHELIA CHAMPACA* L. LEAVES AND STEM BARK IN RATS:

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## Summary

**Objective:** To ascertain the diuretic potential of the leaves and stem bark of *Michelia champaca* L. an Indian medicinal plant widely used for the purpose in traditional systems of medicine. **Methods:** Aqueous extracts of *Michelia champaca* leaves and stem bark dosed at 250 and 500 mg/kg each were administered to adult Swiss albino Wistar rats previously maintained at a uniform water and salt load, and the effect on urinary electrolyte concentrations was determined. The urine output was quantitated at several intervals during the 24 hour collection. **Results:** The higher doses of both the leaves and stem bark aqueous extracts exhibited a more promising diuretic potential. Maximal diuretic response was observed in the 500 mg/kg dose of *Michelia champaca* stem bark aqueous extract, which closely approximated the results for the standard drug Furosemide. Both the doses of stem bark extracts elicited greater volumes of urine and were comparable to that achieved with the standard drug. **Conclusion**: The aqueous extracts of stem bark afforded a better diuretic potential in comparison to the leaves extract, the higher dose evoking pronounced diuresis even greater than the standard Furosemide in terms of $Na^+$ and $K^+$ concentrations and closely approximating Furosemide values in terms of urine volume.

**Keywords:** *Michelia champaca*, diuretic, Stem-bark, leaves

## Introduction

Medicinal plants are very commonly employed in the treatment of certain kidney diseases and many herbs possess significant diuretic activity. [1, 2, 3]The diuretic potential of several herbs used in ethnomedicine as diuretic agents has been confirmed in experimental animals. [4, 2] *Michelia champaca* L. (Magnoliaceae) commonly known as Svarna champa in India is commonly used by many traditional healers (eg. Dr. G. Elias, Reg No. 01323, Leela Vilasam Siddha Varma Vaidyasalai, Karungal, Tamil Nadu) in most of herbal preparations for kidney diseases and diabetes.[5, 6] The plant is also reported to have significant wound healing,[7] antimicrobial,[8] antidiabetic,[9] antitumor,[10] anti-inflammatory,[11] antioxidant,[12] leishmanicidal[13] and anti-infective[14] properties. Traditionally it is used for its astringent, disinfectant, diuretic and cooling properties and in parasitic infections, dysuria and diseases due to vitiated blood.[6] In traditional systems of treatments especially in Ayurveda, the usage of medicinal plants is commonly in their aqueous extracts only. Hence the present study was undertaken to investigate and validate scientifically the status of *Michelia champaca* L. (aqueous extracts of leaves and stem barks) as a diuretic agent as claimed by traditional texts.

## Materials & Methods

**1. Experimental Animals:** Adult Swiss albino Wistar rats of either sex weighing between 200 and 250 g procured from our animal house were housed under standard environmental conditions (25±1°C, 55±5% humidity and 12 h/12 h light/dark cycle). The animals were allowed free access to tap water and standard laboratory rat food. The care and handling of rats were in accordance with the internationally accepted standard guidelines for use of animals, and the protocol was approved by our Institutional Animal Ethics Committee under the CPSCEA. (BBDNITM/IAEC/01/2011)

**2. Plant Material & Preparation of Extracts:** The plant material was collected from local areas of Lucknow, Uttar Pradesh and was authenticated from National Botanical Research Institute, Lucknow by depositing a herbarium (Voucher specimen Ref No. NBRI/CIF/176/2010) and was identified as *Michelia champaca* L. The leaves and stem barks were shade dried at room temperature for more than two weeks and were powdered finely for extraction. 15g of dried powder of leaves was extracted by cold maceration process using 200 mL distilled water. It was mechanically agitated for 6 hours and then allowed to stand for 18 hours. The extract was concentrated to give a semi-solid residue (yield: 16.67%). Powdered stem barks were extracted similarly (yield: 10%).

**3. Standard Drug:** Furosemide injection I.P (Lasix, Aventis Pharma Limited, India), a high ceiling loop diuretic, was chosen as a reference standard.

**4. Experimental Design:**

**4.1. Acute Toxicity Studies:** The acute toxicity studies were performed in accordance with the OECD (Organization for Economic Co-operation and Development) guidelines no. 425 (Up and Down Procedure). [15] No death was observed till the end of the study. The test samples were found safe upto the dose of 2000mg/kg and from the results 500 mg/kg was chosen as the maximum dose for further experimentation.

**4.2. Diuretic Activity:** The diuretic activity was determined following the methods used by Lahlou et al (2007) with slight modifications. [16] Each animal was placed in an individual metabolic cage 24 h prior to commencement of the experiment for adaptation. Rats were fasted overnight with free access to water. Animals were randomly assigned to 6 groups comprising of 6 rats each. Before treatment, all animals received physiological saline (0.9% NaCl) at an oral dose of 5 mL/100 g body weight (BW), to impose a uniform water and salt load.[4] The negative control received distilled water at a dose of 10 mL/kg BW p.o. Positive control received Furosemide at a dose of 10 mg/kg BW p.o.. The remaining 4 groups were dosed at 250 mg/kg and 500 mg/kg BW for leaves and stem bark extracts respectively. Urine was collected and measured at 1, 2, 4, 6, and 24 h after the dose. Sodium and potassium concentrations were determined in the 24 h urine samples. The amount of creatinine excreted in the 24 hour samples was also validated.

**5. Statistical Analysis:** Results are expressed as mean ±S.E.M. Statistical analysis of the data was performed with one-way analysis of variance (ANOVA) (Graph Pad Prism version 3.00, USA)

## Results

The up and down procedure for the acute toxicity studies indicated a reasonably good safety potential for both the parts employed in the study i.e. leaves and stem bark and on this basis 500 mg/kg was chosen as the maximum dose for further experimentation. The experimental data was analysed for the effect on urinary electrolyte concentration, amount of creatinine excreted and the effect on urinary volume. The higher doses of both the leaves and stem bark aqueous extracts exhibited a more promising diuretic potential. Maximum concentrations of the excreted electrolytes ($Na^+$ 189 mEq/24 hours and $K^+$ 56 mEq/24 hours) were achieved with 500mg/kg BW of the stem bark aqueous extract of *Michelia champaca*, which closely approximates the results for the standard drug Furosemide, whereas the aqueous extract of *M.champaca* leaves dosed at 500 mg/kg BW exhibited comparable results with the control group as shown in Table 1. Both the higher doses exhibited creatinine excretion at 1.1 g/24 hours (Table 1) while a trend similar to that seen in effect on urinary electrolyte concentration was observed in case of effect on urine volume, both the higher doses afforded a greater urinary volume as compared to the lower doses. (Table2 and Figure 2) However aqueous extract of *M.champaca* stem bark dosed at 500 mg/kg achieved the maximum urinary volume out the four test doses which also closely approximated the results for the standard drug Furosemide as illustrated in Table 2 and Figure 2.

## Discussion

The most promising diuretic potential of the four test doses as evidenced from the present study was found to be the 500 mg/kg dose of *M.champaca* stem bark aqueous extract, both in terms of urinary electrolyte concentration as well as urinary volume excreted. (Table 1 and Table 2) Amongst the two test doses of the leaves extract, the higher dose afforded better results for urinary electrolyte concentrations. (Table 1 and Figure 1) However both the lower doses of leaves as well as stem bark could not approximate the electrolyte concentrations of the standard drug Furosemide and were also insignificant as compared to the control group. (Table 1)

Although the lower dose of the stem bark aqueous extract exhibited reasonably decent results for the urinary volume excreted, which closely approximated that for the higher dose of the stem bark extract. (Table 2 and Figure 2)
On the basis of the above the following cumulative orders can be traced from this study.
Effect on urinary electrolyte concentration:

$MCS_{500} > F > C > MCL_{500} > MCS_{250} > MCL_{250}$

Effect on urinary volume:

$F > MCS_2 > MCS_1 > MCL_2 = C > MCL_1$

F: Furosemide (Standard), C: Control, $MCS_{250}$: *Michelia champaca* stem barks aqueous extract 250 mg/kg, $MCS_{500}$: *Michelia champaca* stem barks aqueous extract 500 mg/kg, $MCL_{250}$: *Michelia champaca* leaves aqueous extract 250 mg/kg, $MCL_{500}$: *Michelia champaca* leaves aqueous extract 500

| **Treatment** | **Dose (mg/kg BW)** | **Urinary electrolyte concentration** | | **Creatinine excretion (g/24 hours)** |
|---|---|---|---|---|
| | | **$Na^+$ mEq/24 hours** | **$K^+$ mEq/24 hours** | |
| Control (Distilled water) | 10 mL/kg BW | 179 | 42 | 1.02 |
| Furosemide | 10 | 189 | 46 | 0.99 |
| *Michelia champaca* aqueous extract (stem bark) | 250 | 141 | 42 | 0.91 |
| | 500 | 189 | 56 | 1.1 |
| *Michelia champaca* aqueous extract (leaves) | 250 | 139 | 44 | 0.79 |
| | 500 | 169 | 44 | 1.1 |

All values expressed as ± SEM; N = 6; $p \leq 0.05$

**Table 1: Effect of oral doses of the aqueous extracts of *Michelia champaca* (leaves and stem bark) and Furosemide on 24 h urinary electrolyte excretion in Normal rats**

| No. of hours. | Control (Distilled water 10 mL/kg) | Standard (Furosemide 10 mg/kg) | *Michelia Champaca* leaves extract 250 mg/kg | *Michelia Champaca* leaves extract 500 mg/kg | *Michelia Champaca* Stem bark extract 250 mg/kg | *Michelia Champaca* Stem bark extract 500 mg/kg |
|---|---|---|---|---|---|---|
| 1 | 1.5 | 6.0 | 1.5 | 1.5 | 3.0 | 4.5 |
| 2 | 2.4 | 13.5 | 2.4 | 2.7 | 10.5 | 6.0 |
| 4 | 4.5 | 22.5 | 3.0 | 3.6 | 12.0 | 10.5 |
| 6 | 6.0 | 28.5 | 4.5 | 4.5 | 13.5 | 12.0 |
| 24 | 19.5 | 36.0 | 10.5 | 19.5 | 27.0 | 30.0 |

**Table 2: Effect on urine volume on a 24 hour time cycle**

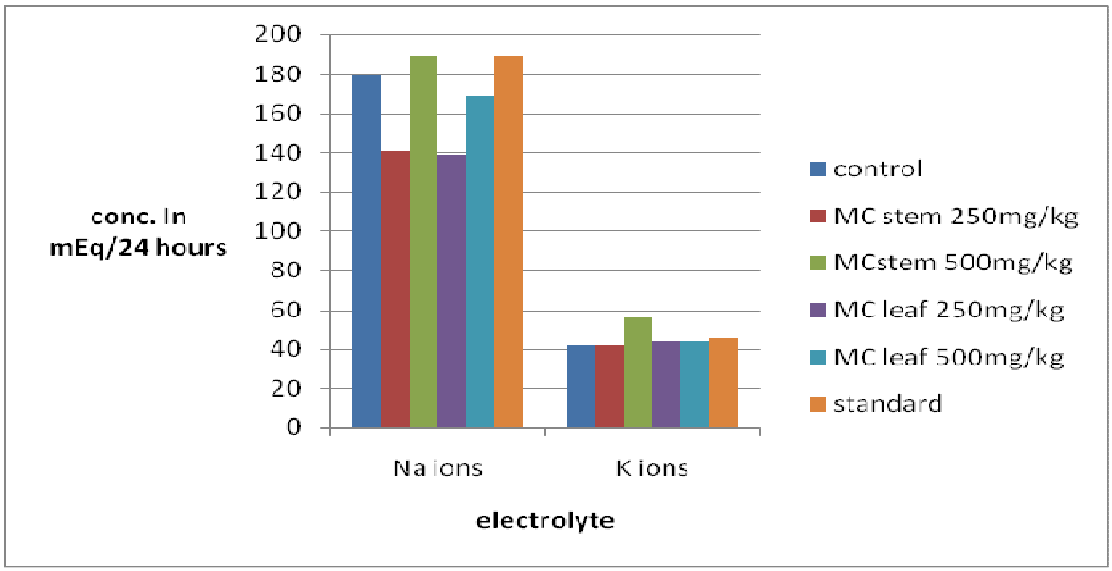


**Fig 1: Effect on urinary electrolyte concentration**

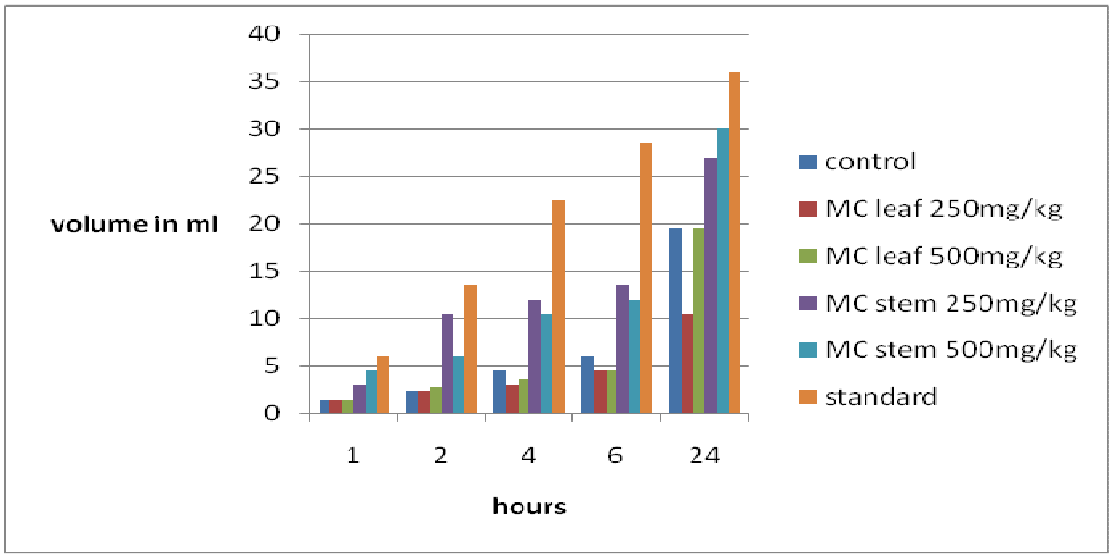


**Fig 2: Effect on urine volume in 24 hour collections**

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**References**

1. Bellakhdar J. La Pharmacop´ee Marocaine Traditionnelle, M´edecine Arabe Ancienne et Savoirs Populaires, Edition Ibis Press, 1997; 150.
2. Maksimovic Z, Dobric S, Kovacevic N, Milovanovic Z. Diuretic activity of Maydis stigma extract in rats. Pharmazie 2004; 59:967–71.
3. Tanira MOM, Ageel AM, Al-Said MS. A study on some Saudi medicinal plants used as diuretics in traditional medicine. Fitoterapia 1988; 5:433–447.
4. Benjumea D, Abdala S, Hernandez-Luis F, P´erez-Paz P, Martin-Herrera D. Diuretic activity of *Artemisia thuscula*, an endemic canary species. J. Ethnopharmacol 2005; 100: 205–09.
5. Nadkarni KM, Nadkarni AK. Indian materia medica. Popular Prakashan, Mumbai: India.
6. Kritikar KR, Basu BD. Indian medicinal plants. Oriental enterprises, Dehradun: India.
7. Dwajani S, Shanbhag TV. *Michelia champaca*: Wound Healing Activity in Immunosuppressed Rats. The Internet Journal of Alternative Medicine. 2009; 7: 1540-45.
8. Khan MR, Kihara M, Omoloso AD. Antimicrobial activity of *Michelia champaca*. Fitoterapia 2002; 73: 744-48.
9. Jarald EE, Joshi SB, Jain DC. Antidiabetic activity of flower buds of *Michelia champaca* Linn. Indian Journal of Pharmacology 2008; 40: 256-60.

10. Hoffmann JJ, Torrance SJ, Wiedhopf RM, Cole JR. Cytotoxic Agents from *Michelia champaca* and *Talauma ovata*: Parthenolide and Costunolide. J Pharm Sci 1977; 66: 883-84.
11. Vimala R, Nagarajan S, Alam M, Susan T, Joy S. Antiinflammatory and antipyretic activity of *Michelia champaca* Linn. (White variety), *Ixora brachiata Roxb*. and *Rhynchosia cana* (Wild.) D.C. flower extract. Indian Journal of Experimental Biology 1997; 35(12): 1310-14.
12. Hasan SMR, Hossain MM, Akter R, Jamila M. DPPH free radical scavenging activity of some Bangladeshi medicinal plants. Journal of Medicinal Plants Research 2009; 3: 875-79.
13. Takahashi M, Fuchino H, Satake M, Agatsuma Y. *In Vitro* Screening of Leishmanicidal Activity in Myanmar Timber Extracts. Biological and Pharmaceutical Bulletin 2004; 27: 921-25.
14. Oumadevi R, Guy R, Francisco ER, Kiban C. Screening for anti-infective properties of several medicinal plants of the Mauritians flora. J Ethnopharmacol 2007; 109: 331-37.
15. W.J.Dixon."The up and down methods for small animals". J.Amer Statist Assoc 1965; 60:967-978.
16. Lahlou S, Tahraoui A, Israili Z, Lyoussi B. Diuretic activity of the aqueous extracts of *Carum carvi* and *Tanacetum vulgare* in normal rats. Journal of Ethnopharmacology 2007; 110: 458–463.