**Evaluation of Analgesic activity of stem bark of *Michelia champaca* Linn.**

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**Abstract**

*Michelia champaca* is also used traditionally to treat various diseases such as microbial infections, inflammations, contraception’s, healing of wounds etc. Further, it was also observed that no characteristic details have been published and carried out in phytochemical analysis on *Michelia champaca* extracts. On the basis of the reported literature review an attempt can be made in development of standardization parameters, physico-chemical analysis on different parts of the plant. Also, the anatomical features will be studied which will be useful in the identification of correct species of *Michelia champaca*. From the literature review it was also observed that so far no any systematic pharmacological screening of the extract of any part of the plant have been carried, therefore attempt can be made in evaluating the species for various pharmacological approaches to prove the efficacy of the plant.

**Keywords:** *Michelia champaca*, Analgesic, leaves

**Introduction**

An analgesic or painkiller is any member of the group of drugs used to achieve analgesia, relief from pain. Analgesic drugs act in various ways on the peripheral and central nervous systems. They are distinct from anesthetics, which temporarily affect, and in some instances completely eliminate, sensation. Analgesics include paracetamol (known in North America as acetaminophen or simply APAP), the non-steroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, and opioid drugs such as morphine and oxycodone. In choosing analgesics, the severity and response to other medication determines the choice of agent; the World Health Organization (WHO) pain ladder specifies mild analgesics as its first step. Analgesic choice is also determined by the type of pain: For neuropathic pain, traditional analgesics are less effective, and there is often benefit from classes of drugs that are not normally considered analgesics, such as tricyclic antidepressants and anticonvulsants.

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**Fig. 1:** *Michelia Champaca*: A Flowering Twing
Despite, the usefulness and importance of the selected species of *Michelia champaca*, accurate information on analgesic activity was not being carried out so far with proper validation and documentation. Therefore, the present work was conceived.

**Material and Methods**

**Selection, collection and authentication of plant/plant material**

The stem bark of plant *Michelia champaca* Linn. parts were collected in the months of Jan-Feb 2017 from the Medicinal garden co Central India Institute of Pharmacy, Indore, M.P. and identified & authenticated by Dr. S. N. Dwivedi, Prof. and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa, M.P. and was deposited in our Laboratory, Voucher specimen No. PCog/MC/10.

**Successive Extraction of Plant Material**

Sample were shattered and screened with 40 mesh. The shade dried coarsely powdered stem bark of *Michelia champaca* (250gms) were loaded in Soxhlet apparatus and was extracted with petroleum ether (60-62°C), Chloroform, ethanol and water until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined.

**Pharmacological Screening**

**Procurement of experimental animals**

The mice were used for acute toxicity study as per OECD guidelines 423. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee after scrutinization.

**Evaluation of analgesic activity**

**Animals**

Female Wistar rats of (200-250 gm) were procured from Veterinary College, Mhow, Indore, (M.P.) maintained under ideal feeding and management practices in the laboratory. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee after scrutinization.

**Study Design**

**Hot plate**

Animals were divided into V groups, each group containing six animals each. Group I served as the positive control with no protection. Group II animals received the standard drug of Indomethacine 5 mg/kg body weight, whereas group III to V animals were orally administered the various plant extracts viz., ethanolic and aqueous extracts at the dose of 250 and 500 mg/kg body weight respectively. The temperature of the hot plate was maintained 55±1 °C, mice were placed on the hot plate and time in seconds for paw licking or jumping was recorded as basal reaction time. Cut off time in the absence of response was 15sec to prevent the animals being burnt. The reaction time in seconds (latency period) was observed on hot plate, the time taken for mouse to react to the thermal pain by licking its paw or attempting to jump out. Observations were made before and after administration of respective drugs at an interval of 60 min.

**Tail Flick Method**

The animals were tested for tail flick by Analgesiometer (Techno Electronics, Lucknow, India) as it was described earlier (Miranda et al, 2003)21 . The basal time was noted at first for each animal. Current through the naked nichrome wire was set at 5 Amp over which 1-2 cms from the tip of the tail was exposed to check out the response. The cut off time was set at 10 sec to prevent any tissue damage. The time (in second) required for the animal to withdraw (flick) its tail from the heat source was measured. The reaction time was noted in minutes after the animals were treated orally with various doses of extract and with Indomethacine (5 mg/kg). Normal saline (0.1ml/10gm) served as control group.

**Statistical analysis**

All the values were statistically analyzed by one-way analysis of variance (ANOVA) followed Bonferroni’s post hoc test. Comparison between control and drug treated groups were considered to be significant (*P<0.01). All values are expressed as mean ± SEM.

**Results and Discussion**

The present work carries the results of ‘Evaluation of Analgesic activity of stem bark of *Michelia champaca* Linn. It indicates the quality standards and utilization of selected plants for the treatment of various ailments among the inhabitants as mentioned in folk-lore and to validated scientifically.
Acute Toxicity Studies of Extracts
The aqueous and ethanolic extracts of stem bark of *M. champaca* were screened for acute toxicity study by OECD guideline no. 423 for determination of LD$_{50}$. The results showed that the aqueous and ethanolic extracts i.e., AEMCSB and EEMCSB were belonging to category-5 (unclassified). Hence, LD$_{50}$ was 5000 mg/kg, therefore, ED$_{50}$ was 500 mg/kg. Therefore, two doses of 200 and 400 mg were selected for present investigation. The results were presented in table 1.

Table 1: Determination of LD$_{50}$ and ED$_{50}$ of aqueous and ethanolic extract of *M. champaca* Stem bark

<table>
<thead>
<tr>
<th>S/N o.</th>
<th>No. of Animals</th>
<th>Extract Dose (mg/kg)</th>
<th>No. of death of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AEMCSB</td>
<td>EEMCSB</td>
</tr>
<tr>
<td>1.</td>
<td>3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>3</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>3</td>
<td>300</td>
<td>0</td>
</tr>
<tr>
<td>4.</td>
<td>3</td>
<td>2000</td>
<td>0</td>
</tr>
<tr>
<td>5.</td>
<td>3</td>
<td>5000</td>
<td>0</td>
</tr>
</tbody>
</table>

Evaluation of analgesic activity

**Hot Plate Method**
The present investigation reveals that the ethanolic extract (EEMCSB) of *M. champaca* exhibit its maximum analgesic activity of 74.79%, by hot plate method at the given dose of 500mg/kg, followed by 71.28% EEMCSB at the dose 250 mg/kg, and it was significant when compared with control and standard group.

The aqueous extract showed a moderate analgesic activity when compared with control and standard group. The results were presented in table 2.

**Tail Flick Method**
The present investigation reveals that the ethanolic extract of *M. champaca* exhibit its maximum analgesic activity by tail flick method at the given dose of 500mg/kg, and it was significant when compared with control and standard group.

The aqueous extract showed a moderate analgesic activity when compared with control and standard group. The results were presented in table 2.

Table 2: Analgesic effect of aqueous and ethanolic extract of *M. champaca* Stem bark

<table>
<thead>
<tr>
<th>Group</th>
<th>Reaction time (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hot Plate</td>
</tr>
<tr>
<td></td>
<td>Pre treatment</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>11.8 ± 0.8</td>
<td>11.1 ± 0.7</td>
</tr>
<tr>
<td>Standard 5mg/kg</td>
<td>10.5 ± 1.2</td>
</tr>
<tr>
<td>AEMCSB 250 mg</td>
<td>11.5 ± 0.3</td>
</tr>
<tr>
<td>AEMCSB 500 mg</td>
<td>11.9 ± 0.7</td>
</tr>
<tr>
<td>EEMCSB 250 mg</td>
<td>11.4 ± 0.2</td>
</tr>
<tr>
<td>EEMCSB 500 mg</td>
<td>10.9 ± 1.2</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S.E.M (n=6), ***P<0.001 as compared control, **P<0.01 as compared control, One-way ANOVA followed by Bonferroni multiple comparison test.
Graph 1: Analgesic effect of aqueous and ethanolic extract of *M. champaca* Stem bark by Hot plate method

Graph 2: Analgesic effect of aqueous and ethanolic extract of *M. champaca* Stem bark by Tail flick method

**Conclusion**

Nature has been a source of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Several species of medicinal plant are used in the treatment of human disease and disorder. Medicinal plants generated commercial demand for pharmacopoeial drugs and their products in India. Efforts have been made in recent years to introduce many of these drug plants to common people. It is evident that many valuable herbal drugs have been discovered by knowing that a particular plant was used by the ancient folk healers for the treatment of some kind of ailments. Moreover, the medicinal plant wealth are our national heritage and it seems to...
be the first and foremost line of defense for the treatment of various diseases mostly tribal and rural communities and is a worth scientific study. From the literature review it was revealed that till date no any systematic work done on accessing analgesic activity of stem bark of Michelia champaca, therefore the present work was conceived. This work comprises of ‘Evaluation of Analgesic activity of stem bark of Michelia champaca Linn.’. The parameter employed for this purpose includes development of standardization parameters as per WHO Guidelines, successive extraction of plant material, preliminary phytochemical screening and pharmacological activities (analgesic).

In current scenario, herbs are the potent sources of medicines used in the treatment of various disease and disorders. Since, plants are used as medicine there is prompt need of evaluation of plant species, therefore, the present work was conceived to evaluate the phytochemical and pharmacological screening of few Indian medicinal plants. The Pharmacognostical evaluation of Indian medicinal plants M. champaca Linn. was studied which include the morphological, power microscopy and physicochemical studies. The morphological studies of species plant part were studied which will be beneficial for the validation and assessment of quality control parameters of these plants to find out the presence of adulterant if any in order to establish the quality, safety and efficacy. From the data of physicochemical analysis it was concluded that the plants has optimum level of carbon content which was establish by the ash content data. Other parameter so found was within the limit as per WHO guideline for standardization of Medicinal plants. The percentage yield value of various extracts was estimated and repoted. From the results of preliminary phytochemical screening it was concluded that the aqueous and ethanolic extracts contained various phytochemicals such as alkaloids, glycosides, saponins, carbohydrates etc.whereas pet ether and chloroform extract have very less phytochemicals and based on the same for the pharmacological studies only two extract ethanolic an aqueous were chosen. The aqueous and ethanolic extracts of plants were screened for acute toxicity study by OECD guideline no. 423 for determination of LD$_{50}$. The results showed that the aqueous and methanolic extracts were belonging to category-5($>$2000-5000). So, LD$_{50}$ was 2500 mg/kg, therefore, ED$_{50}$ was 250 mg/kg for all the extracts. The present investigation reveals that the ethanolic extract (EEMCSB) of M. champaca exhibit its maximum analgesic activity of 74.79%, by hot plate method at the given dose of 500mg/kg, followed by 71.28% EEMCSB at the dose 250 mg/kg, and it was significant when compared with control and standard group.

The aqueous extract showed a moderate analgesic activity of AEMCSB 35.48 % at the dose 500 mg/kg and AEMCSB 32.13 % at the dose 250 mg/kg when compared with control and standard group. The present investigation reveals that the ethanolic extract of M. champaca exhibit its maximum analgesic activity of by tail flick method at the given dose of 500mg/kg, and it was significant when compared with control and standard group.

The aqueous extract showed a moderate analgesic activity when compared with control and standard group. Hence, from the present work it was concluded that the selected medicinal plants M. champaca Linn. of Indian origin possess optimum analgesic activity which will claims their folk-lore uses as mentioned in traditional system of medicine.

References