

Application of Magnetic Stirrer for Influencing Extraction Method on *Tectona grandis* as Analgesic Activity

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ABSTRACT

The aim of the current study was to show the application of magnetic stirrer for influence of extraction methods on plant material. To achieve maximum possible extraction efficiency, it becomes necessary to optimize the extraction methodology. Magnetic stirrer, which possess certain advantages, these are, the reduction in organic solvents consumption, improvement in extraction efficiency. *Tectona grandis* is commonly known as "teak" belongs to verbenaceae family. The whole plant is medicinally important and many reports claim to cure several diseases according to Indian traditional system of medicines. The different extracts from various parts of teak shows expectorant, anti-inflammatory, anthelmintic properties. In proposed work apply magnetic stirrer for influencing extraction method to evaluate analgesic activity on *Tectona grandis* extract.

Keywords: *Tectona grandis*

INTRODUCTION

There are certain general techniques of medicinal plant extraction used in different areas such as Pharmaceutical industries, herbal industries, research laboratories include Soxhlet extraction ultrasonic extraction, microwave-assisted extraction & magnetic stirrer. Among them magnetic stirrer possess advantages the reduction in organic solvents consumption, improvement in extraction efficiency. A magnetic stirrer is equipment used to create rotating magnetic field. It is designed such that there is a small bar magnet and stand or plate containing the rotating magnet. In general, the bar magnet is coated with plastic and plate contains rotating magnet. It is possible to create a rotating magnetic field with the help of a rotating magnet. There are several types of magnetic stirrer available and it all depends on your selection of size, application and configuration. Extracting methods and solvents are important for quantity and quality of the extracts. Appropriate extraction method for each plant should be investigated in order to promote the higher amount of active components^{1,2}.

Classic techniques for solvent extraction of active constituents from medicinal plant matrices are based on the choice of solvent coupled with the use of heat or agitation. Soxhlet extraction is a general and well-established technique, which surpasses in performance other conventional extraction techniques except for, in limited fields of application, the extraction of thermo labile compounds.

Advantages and Disadvantages of Soxhlet Extraction

Advantages

The displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with the solid matrix.

Maintaining a relatively high extraction temperature with heat from the distillation flask.

No filtration of the extract is required.

Disadvantages

Agitation is not possible in the Soxhlet device.

The possibility of thermal decomposition of the target compounds cannot be ignored as the extraction usually occurs at the boiling point of the solvent for a long time⁶.

Tectona grandis is commonly known as "teak" belongs to verbenaceae family. The whole plant is medicinally important and many reports claim to cure several diseases according to Indian traditional system of medicines. The uses of traditional medicinal plants for primary healthcare have steadily increased worldwide in recent years. Traditional plant medicines serve as a source of various types of active principle and WHO estimates 70 % of the World population still relies on the herbal medicines. Out of the total 2, 25,000 species of plants, only less than 10 % have been studied so far for their medicinal uses. The plant under investigation is *Tectona grandis* survey reveals that the plant is used in the treatment of Urinary discharge, bronchitis, cold and headache. In scabies, used as a laxative and sedative, as diuretic, anti diabetic, analgesic and anti-inflammatory. In the present study we isolated 19 phytochemicals qualitatively from various extracts. Isolated 19 secondary metabolites from the leaves extract of *Tectona grandis* Linn. Namely Steroids, Tannin,

Table 1: Taxonomy of *T. grandis*.

Kingdom	Plantae
Super division	Angiosperms
Division	Eudicots
Class	Asterids
Order	Lamiales
Family	Verbenaceae
Genus	<i>Tectona</i>
Species	<i>Grandis</i>

Saponin, Anthocyanin, Coumarins, Emodins, Alkaloids, Proteins, Amino Acids, Carbohydrate, Flavonoids, Diterpenes, Physterol, Phenol, Phlobatannin, Leucoanthocyanin, Anthraquinone, Cardial Glycosides and Chalcones³.

This work was part of the scientific validation of the ethno pharmacological claim about the analgesic and properties of leaves extracts. To the best of our knowledge, there are no reports of leaves bark extract of *T. grandis* as analgesic. Hence, we evaluated the analgesic activities of ethanol & chloroform mixture as solvent extracts of *T. grandis* in Albino Wistar rats using hot-plate. The proposed work evaluate analgesic activity on *Tectona grandis* leaves by applying magnetic stirrer as extraction method for influencing extraction. Ethanol & chloroform used as solvent in 6:4 proportion.

MATERIAL AND METHODS

Collection of Plant Material

The plant leaves were collected from Sahyadri College of Pharmacy Methwade Campus area and authenticated by local Botanist from Sangola Mahavidyalay Sangola . The plant materials were washed under running tap water to remove the surface pollutants and the air dried under the shade. The dried sample was powdered and used for further studies.

Magnetic stirrer extraction (ME)

Five gram of fine powder of plant material was extracted with 100 ml of an appropriate solvent in a round bottom flask with magnetic stirrer for 24 hours at room temperature. The leaves extract were then centrifuged at 5000 rpm for 15 min. An external magnetic field is applied to the magnetic stirrer to mix the solution which facilitates the rotating of the small magnetic bar placed in the mixture of interest.

Solvent

Ethanol & chloroform mixture used as solvent in 6:4 proportion of ratio.

Selection of Experimental Animals

Wistar albino rats weighing between 200-250 g were procured from animal house, Sahyadri College of Pharmacy, Methwade. They were acclimatized for one week to the laboratory condition in well ventilated room at temperature $25 \pm 2^\circ\text{C}$ and relative humidity of 30-70% with a 12:12 light-dark cycle, and fed with standard pellet supplied by Hindustan lever. Co. Mumbai with water *ad libitum* throughout the course of study. The animals were fasted for 18 h prior to the experiment. All the animal experiments were conducted according to the ethical norms approved by CPCSEA, and ethical clearance was

granted by institutional ethical animal committee. The present study was conducted in Dept. of Pharmaceutical Chemistry Sahyadri College of Pharmacy, Methwade.

Experimental Design

Twenty Five albino rats of either sex were taken and divided into 4 groups, each consisting of 5 rats each. Drugs were administered to all the groups (control, tests and standard) through p.o. route, Sodium carboxyl methyl cellulose (CMC) did not produce evident changes in activity response.

Group I (control group): 0.5% sodium CMC in distilled water at 10 mL/kg body weight.

Group II (standard group) for analgesic activity: Diclofenac Sodium (10 mg/kg) suspension in 0.5% sodium CMC served as standard drug at 10 mL/kg body weight.

Group III (EETG 250 mg/kg b.w) suspension in 0.5% sodium CMC (250 mg/kg) at 10 mL/kg

Group IV (EETG 500 mg/kg b.w) suspension in 0.5% sodium CMC (500 mg/kg) at 10 mL/kg.

Preparation of drugs and Chemical solutions

EETG (250mg/kg body weight) was dissolved in sufficient quantity of solvent in normal saline and use in the treatment. EETG (500mg/kg body weight) and Diclofenac Sodium (10mg/kg body weight) was dissolved together in sufficient quantity of solvent (normal saline) & Sodium CMC was prepared by using normal saline of strength of 1% v/v.

Acute oral toxicity studies

Acute oral toxicity studies were performed according to OECD-423 guidelines (acute toxic class method). Wistar Albino rats ($n = 3$) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. The Ethanolic extract of *T. grandis* was administered orally at a dose of 5 mg/kg initially and mortality was observed for 3 days. If mortality was observed in 2/3 or 3/3 animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one rat out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher doses such as 50, 300 and 2000 mg/kg⁵.

Evaluation of Analgesic Activity

Hot Plate Method

Groups of Wistar Albino Rats of either sex weighing between 200-250g were used. Rats were placed on a hot plate maintained at $55 \pm 0.5^\circ\text{C}$. The reaction time was taken as the interval from the instant animal reached the hot plate until the moment animal licked its feet or jumped out. A screening was done and only those rats which react in 15s were selected to avoid thermal injury. The latency is recorded before and after 15, 30, 45, 60 and 90 min following oral administration of the test compounds and the standard drug. The rats was not placed on hot plate for more than 15 seconds to avoid thermal damage to the paw.

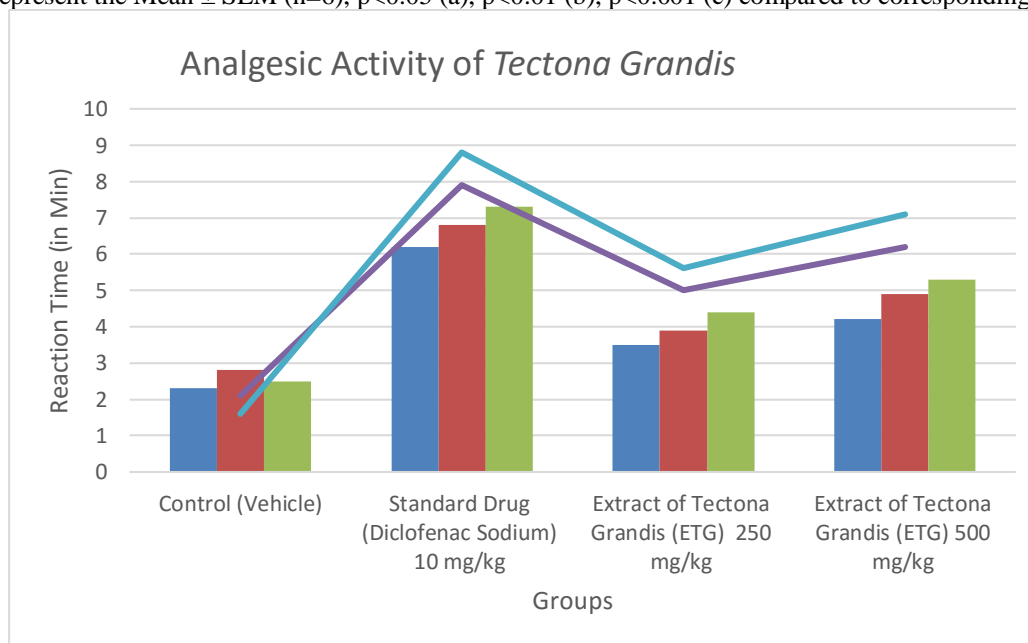
Statistical Analysis

All the results were expressed as mean \pm standard error mean (S.E.M.). Data were analyzed using one-way ANOVA followed by Dunnett's t-test. The analysis was

Table 2: Analgesic effect of ethanolic extract of *Tectona Grandis* (250 & 500mg/kg), leaves on heat stimulating response in the hot plate test in Wistar albino rats.

Groups	Dose (mg/kg)	Reaction Time (in Min)				
		15	30	45	60	90
Control (Vehicle)	10 mL/kg	2.3 ± 0.4 ^b	2.8 ± 0.2 ^a	2.5 ± 0.5 ^c	2.1 ± 0.1 ^a	1.6 ± 0.2
Standard Drug (Diclofenac Sodium)	10 mg/kg	6.2 ± 0.2 ^a	6.8 ± 0.6 ^a	7.3 ± 0.4 ^b	7.9 ± 0.2 ^b	8.8 ± 0.3 ^c
Extract of <i>Tectonia Grandis</i> (ETG)	250 mg/kg	3.5 ± 0.1 ^b	3.9 ± 0.2	4.4 ± 0.5 ^a	5.0 ± 0.1 ^c	5.6 ± 0.7 ^a
Extract of <i>Tectonia Grandis</i> (ETG)	500 mg/kg	4.2 ± 0.5 ^c	4.9 ± 0.2 ^b	5.3 ± 0.7 ^a	6.2 ± 0.6 ^b	7.1 ± 0.5 ^c

The data represent the Mean ± SEM (n=6), p<0.05 (a), p<0.01 (b), p<0.001 (c) compared to corresponding control.

Figure 1: Analgesic Activity of *Tectona Grandis* Plant Extracts.

carried out using Graph pad software. P < 0.05 was considered as statistically significant.

RESULT AND DISCUSSION

The result of the hot plate method is given in [Table 2]. From 15 min to 90 min it was found that the group ranks were significantly ($P < 0.01$, $P < 0.05$, $P < 0.001$) different. At each time interval from 15 min onward the standard drug diclofenac sodium showed the highest mean rank ranging from 6.2 to 8.8, whereas ETG at 500 mg/kg was the second mean rank ranging from 4.2 to 7.1 following diclofenac. The results showed that there is no significant ($P > 0.05$) difference between the two from 15 min to 90 min. In Eddy's hot plate test the predicted onset time of ETG was found to be at 45 min. The results of this study showed that *T. grandis* can be effective in analgesic disorder.

For evaluating any specific property in plant extracts, selection of most appropriate extraction method is required because all the methods and solvents differ in mechanism of extraction from each other. Any one method cannot be said as universally applicable for extraction of all types of bioactive metabolites. To achieve maximum possible extraction efficiency, it becomes necessary to optimize the extraction methodology. Magnetic stirrer, which possess certain advantages, these are, the reduction in organic

solvents consumption, improvement in extraction efficiency.

CONCLUSION

As per the given data showed, it is concluded that, magnetic stirrer apply for influence of extraction methods on plant material. Magnetic stirrer influence extraction efficiency of *T. grandis*. The study showed that there is no significant ($P > 0.05$) difference between the two from 15 min to 90 min. The study showed that *T. grandis* effective in analgesic disorder.

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