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Antinociceptive Properties of Dichloromethane:Methanolic Leaf Extracts of Caesalpinia volkensii and *Maytenus obscura* in Animal Models

Mwangi B. Maina*, Gitahi S. Maina, Njagi J. Muriithi, Mworia J. Kiambi, Aliyu Umar, Mwonjoria K. John, Ngure G. Mutero, Juma K. Kelvin, Njoroge W. Ann, Mburu N. David and Ngugi M. Piero

Department of Biochemistry and Biotechnology, Kenyatta University, P.O Box 43844-00100, Nairobi, Kenya

***Corresponding author:** Mwangi B. Maina, Department of Biochemistry and Biotechnology, Kenyatta University, P.O Box 43844-00100, Nairobi, Kenya, Tel: +254 725 619 448; E-mail: mwangi.bonifacemaina@gmail.com

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Abstract

The purpose of physiological pain is protection. Pain is associated with high morbidity and socioeconomic burden. Conventional medication of pain is expensive and arguably associated with various severe adverse effects hence the need to develop herbal agents that are effective as alternative. This study was designed to evaluate the antinociceptive of *C. volkensii* and *M. obscura* growing in Embu County, Kenya. Experimental animals were divided into four groups; normal group, diseased negative control group, diseased reference group and diseased experimental groups. Pain was induced into rats using formalin. The experimental groups were treated with leaf extracts of the plants at dose levels of 50 mg/kg, 100 mg/kg and 150 mg/kg. Anti-nociceptive activities in rats were compared with diclofenac (15 mg/kg) as the standard conventional drug. In antinociceptive study, the leaf *extract C. volkensii* reduced pain by between 6.82-15.24% only in the early phase while the leaf extracts of *M. obscura* reduced pain by between 12.39-34.81% (in the early phase) and between 6.4-12.4% (in the late phase). Diclofenac reduced pain by between 7.58-9.66% (in the early phase) and by 69.87% in the late phase. Further, the phytochemical screening results showed that the extracts of *C. volkensii* had flavonoids, steroids and phenolics while the leaf extracts *M. obscura* had phenolics, terpenoids and saponins. flavonoids. Therefore, the study has established that the DCM: methanolic leaf extracts of *Caesalpinia volkensii* and *Maytenus obscura* are effective in management of pain.

Keywords: *Caesalpinia volkensii*; *Maytenus obscura*; Leaf extracts

Introduction

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage [1] and acts as a warning signal against disturbances either in the body or in the external environment of an individual. The objective of the treatment of pain is to remove or abolish the cause of pain. Analgesics such as non-steroidal anti-inflammatory drugs, opioids and antidepressant are used for treatment of pain [2]. Some of the pain mediators produced act as neuro modulators, generating sustained activation and sensitization of primary nociceptors and higher-order neurons involved in the transmission of pain [3]. Almost all pharmacological treatments may produce side effects [4]. Alternative complementary treatments are increasingly being used to alleviate affective disorders and there are claims that they are safer and much effective than conventional drugs [5].

Analgesic substances have been purified from plants, resulting in the identification of novel agents with known mechanism of actions. Natural products still hold a great promise for the future of drug discovery in the treatment of pain disorders [6]. The study aimed at providing preliminary information of producing standardized herbal formulation from the DCM: methanolic leaf extracts of *C. volkensii* and *M. obscura* that is more effective in the treatment of pain that are arguably less toxic, less costly and easily accessible than antinociceptive drugs.

The *C. volkensii* plant is used in Kenya and Tanzania mostly to treat malaria. In the area around Nairobi (Kenya) herbalists prescribe a decoction of leaves to cure malaria. The leaf decoction is also taken to fight pains during pregnancy. Pregnant women take powdered pods dissolved in water to relieve stomachache. Roots are eaten cooked, raw or as an addition to palm wine for their aphrodisiac properties, also used to treat gonorrhea and bilharzia. Seeds are used to cure stomach ulcers. Flower buds are crushed and applied to the eye to treat eye problems. *C. volkensii* leaf extracts have shown to lower blood glucose on allaxon induced diabetic mice [7]. Species belonging to genus Maytenus are widely used in the folk medicine such as antiseptic, antiasthmatic, fertility-regulating agents, antitumor and antiulcer [8].

Materials and Methods

Collection and preparation of plant materials

The plants were collected from Siakago division, Mbeere North Sub County, Embu County, Kenya. The fresh leaves of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A. Rich.) were identified and collected with the help of local traditional medicinal practitioners. The plant samples were provided to an acknowledged taxonomist for botanical authentication and a voucher specimen deposited at the Kenyatta University Herbarium. The samples were collected with acceptable bio-conservation methods, sorted out, cleaned, and transported in polythene bags to the Biochemistry and Biotechnology laboratories of Kenyatta University for studies.

Sample processing and extraction

The leaves of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A.Rich.) were chopped into small pieces and air dried at room temperature for two weeks until they were properly dry. They were then ground into fine powder using an electric mill followed by sieving through mesh sieve. For each sample, 200 grams of powder was weighed, soaked separately in a cold 1:1 mixture of methanol and DCM and stirred for six hours to obtain the extract. The extract was filtered using Whatman filter papers and the filtrate concentrated under reduced pressure and vacuum using rotary evaporator. The concentrate was put in airtight container and stored at -4°C before use in bioassays.

Experimental Design

Laboratory animals

Wister rats of either sex, between 2-3 months old and weighing 140–180 gm were used in the study [9]. The animals breeding colonies were acquired and bred in the animal breeding and experimentation facility of the department of Biochemistry and Biotechnology, Kenyatta University. The animals were be kept in the standard cages and maintained under the standard laboratory conditions of ambient room temperature with 12 hr light followed by 12 hr dark cycle throughout the experiments. They were fed on a standard rodent pellets diet and supplied with water *ad libitum* [10].

The animals were allowed to acclimatize for 48 h before beginning the experiment. The ethical guidelines and procedures for handling experimental animals were followed and all experimental protocols were in compliance with the institutional ethics committee on research in animals as well as internationally accepted principles for laboratory animal use and care throughout the study. All the tests were carried out during the daytime in a quiet laboratory setting with ambient illumination and temperature similar to those of the animal house.

Evaluation of antinociceptive activity

The animals were selected 24 h prior to experimentation on the basis of their normal reaction (Licking time) to the formalin test. The experimental animals were divided into six groups of five (n=5) and treated as shown in Table 1.

Group	Status	Treatment
I	Normal control	2.5% formalin only
II	Negative control	2.5% formalin+10% DMSO
III	Positive control	2.5% formalin+15 mg/kg diclofenac+10% DMSO
IV	Experimental group A	2.5% formalin+50 mg/kg+10% DMSO
V	Experimental group B	2.5%formalin+100 mg/kg+10% DMSO
VI	Experimental group C	2.5% formalin+150 mg/kg+10% DMSO

Table 1: Treatment protocol for evaluation of antinociceptive activities.

The formalin test was carried out as described in ref. [11] where all the animals were injected with 0.1 ml of 2.50% formalin in their left dorsal hind paw to induce nociceptive behaviour of lifting, licking and biting12. Thirty minutes following the treatments, the animals were injected with formalin. The time that the rats spent lifting, licking or biting the injected paw were recorded according to the described response pattern described in ref. [12]. The test was carried out in a transparent plastic chamber (30×30×30) cm with a mirror placed at the bottom of the chamber to allow an unobstructed view of the rats. Two distinct periods of intensive nociceptive activity were identified and scored separately. The first period (Early Phase) was recorded 1-5 minutes after formalin injection and the second period (Late Phase) was recorded 20-40 minutes after formalin injection. The percentage inhibition of the licking was then calculated using the following formula;

C-T ×100/C

Where; C=the vehicle treated control group value for the each phase.

T=the treated group value for each phase.

Qualitative phytochemical screening

The extracts obtained were subjected to qualitative phytochemical screening for presence or absence of selected chemical constituents using protocols described in refs. [13,14]. Secondary metabolites tested for include; flavonoids, phenolics, saponins, alkaloids, cardiac glycosides, steroids and terpenoids.

Data management and statistical analysis

The experimental data on the Licking time/Latency of pain response was obtained from all the animals in different groups, recorded and tabulated on a broad sheet using MS Excel program. The results were expressed as mean ± standard error of mean (SEM) for analysis. Statistical significance of difference among group were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests to separate the means and obtain the specific significant differences among the different groups. Unpaired student t-test was done to compare the mean activities of leaf extracts of *Caesalpinia volkensii* and *Maytenus obscura*. The value of $P \leq 0.05$ was considered to significant. Analysis of the data was done using Minitab statistical software.

Results

Antinociceptive effects of DCM: Methanolic extract of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A Rich.) Cuf. on formalin induced pain in rats

Formalin induces pain in two phases; early phase which takes one to five minutes and the late phase which occurs fifteen to thirty minutes after formalin injection. Generally, the DCM: Methanolic leaf extracts of *C. volkensii* reduced formalin induced pain in rats only in the early phase, which was indicated by reduction in paw licking time (Table 2).

In the early phase, the percent inhibition of paw licking time upon administration of DCM:Methanolic leaf extracts of *C. volkensii* at the three dose levels (50, 100 and 150 mg/kg body weight) were -3.27%, 6.82% and 15.24%, respectively (Table 2 and Figure 1). The treatment of rats with DCM: Methanolic leaf extracts of *C. volkensii* at the dose

level of 150 mg/kg body weight significantly reduced paw licking time compared to control groups ($p<0.05$; Table 2). The antinociceptive effectiveness of DCM: Methanolic leaf extracts of *C. volkensii* at the dose levels of 100 and 150 mg/kg body weight was comparable to that of diclofenac ($p>0.05$; Table 2).

In the late phase, the DCM: Methanolic leaf extracts of *C. volkensii* at the three dose levels (50, 100 and 150 mg/kg body weight) reduced formalin induced pain in rats by -197.5-186.4% and -185.9%, respectively (Figure 1). In this phase, the antinociceptive effectiveness of DCM: Methanolic leaf extracts of *C. volkensii* at all the three dose levels of was not significantly different from baseline and negative control groups ($p>0.05$; Table 2). Therefore, the extracts did not exhibit significant antinociceptive activity compared to diclofenac (positive control) ($p<0.05$; Table 2).

On the other hand, DCM: Methanolic leaf extract of *M. obscura* at the three dose levels (50, 100 and 150 mg/kg body weight) generally reduced formalin induced pain in both phases, which was indicated by reduction in paw licking time (Table 2 and Figure 1).

In the early phase, the antinociceptive effectiveness of DCM: Methanolic leaf extract of *M. obscura* at the dose levels of 50 mg/kg and 150 mg/kg body weight were not significantly different from each other and the control groups ($p<0.05$; Table 2). However, the antinociceptive activity of DCM: Methanolic leaf extract of *M. obscura* at the dose level of 100 mg/kg body weight was significantly different compared to control groups ($p>0.05$; Table 2).

In the late phase, DCM: Methanolic leaf extract of *M. obscura* at the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) reduced formalin-induced paw licking time by 12.4%, 6.4% and 5.39%, respectively. The antinociceptive effectiveness of DCM: Methanolic leaf extract of *M. obscura* at all dose levels (50, 100 and 150 mg/kg body weight) were not significantly different from each other, baseline and negative control groups ($p<0.05$; Table 2). However, diclofenac was significantly more effective than the three dose levels (50, 100 and 150 mg/kg body weight) ($p<0.05$; Table 2).

In comparison, the DCM: Methanolic leaf extract of *M. obscura* exhibited more effective antinociceptive effect than *C. volkensii* at all the dose levels (50, 100 and 150 mg/kg body weight) in both early and late phases of formalin test period (Figures 1 and 2).

Groups	Treatment	Phase 1	Phase II
Baseline	Formalin	251.01 ± 09.45^{ab} -5.45%	(-07.46%)
Negative control	DMSO	266.00 ± 06.82^{ab} 0.00%	760.00 ± 46.70^{b} 0.00%
Positive control	Diclofenac	240.40 ± 08.07^{ab} -9.66%	462.60 ± 14.16^{a} (-73.83%)
DCM: Methanolic Leaf extracts	50 mg/kg	274.00 ± 07.12^{a} (-3.27%)	795.00 ± 59.99^{b} (-197.50)
	100 mg/kg	246.40 ± 19.58^{ab} -6.82%	758.00 ± 42.37^{b} (-186.4%)
	150 mg/kg	226.00 ± 11.77^{b} -15.24%	758.00 ± 11.77^{b} (-185.9%)

Values are expressed as Mean ± SEM for five animals per group. Statistical comparison were made within a column and values with the same superscript are not significantly different by ANOVA followed by Tukey's post hoc test ($p>0.05$). Figures in paranthesis indicate percent paw licking inhibition. Formalin=2.5%; DMSO=10%; Diclofenac=15 mg/kg.

Table 2: The antinociceptive effects of DCM: Methanolic leaf extracts of *Caesalpinia volkensii* Harms in rat model.

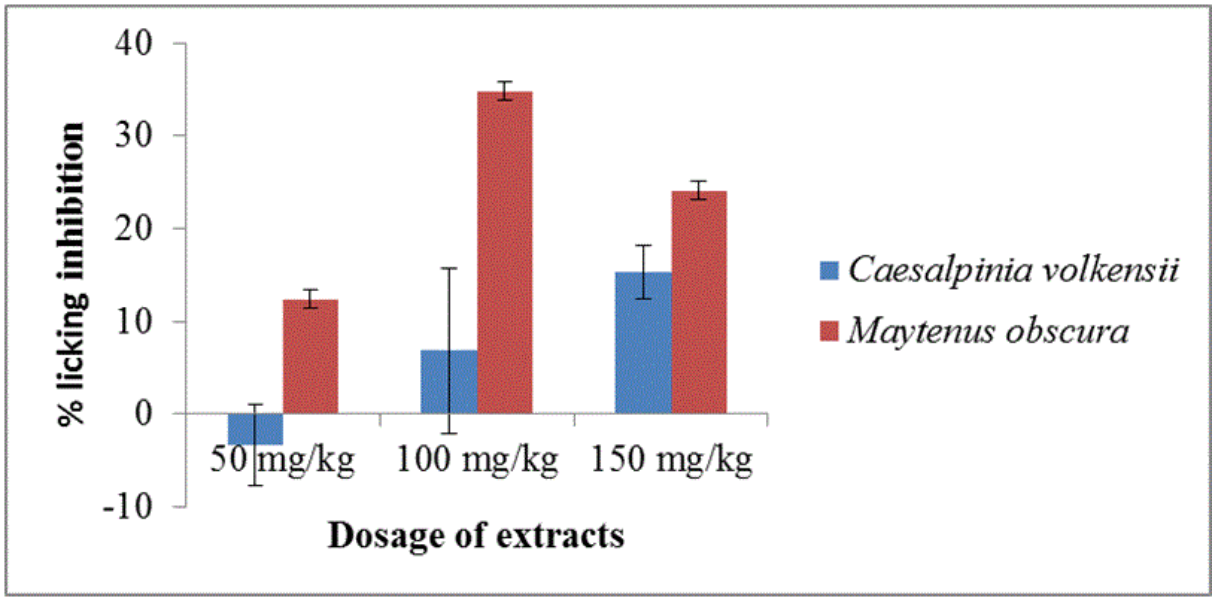

Figure 1: Percent licking inhibition by DCM: Methanolic leaf extracts of *C. volkensii* and *M. obscura* in phase 1 of the formalin test.

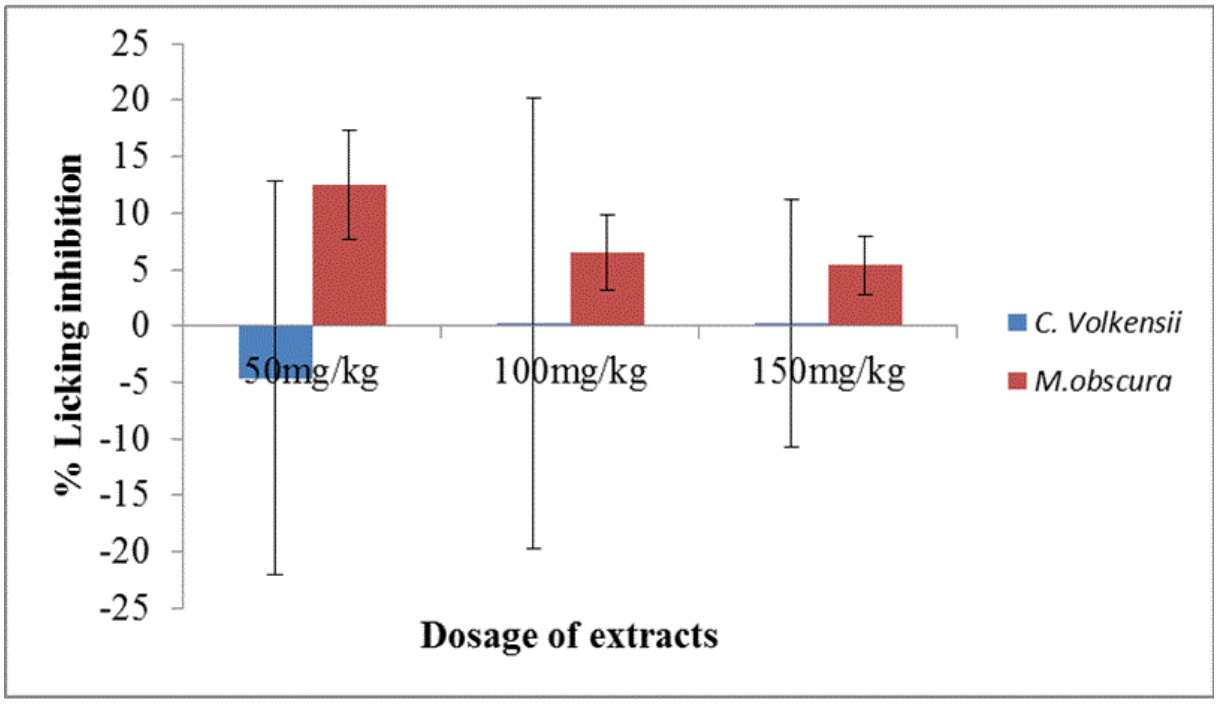

Figure 2: Percent licking inhibition by DCM: Methanolic extracts of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A. Rich.) in phase 2 of the formalin test.

Phytochemical screening

As Table 3 shows, *Caesalpinia volkensii* contained flavonoids, steroids and phenolics whereas, alkaloids, terpenoids, saponins and cardiac glycosides were absent.

Groups	Treatment	Phase I	Phase II
Baseline	Formalin	257.00 ± 09.44^{b} (06.27%)	863.80 ± 18.87^{b} (-1.84%)
Negative control	DMSO	274.80 ± 05.91^{b} (00.00%)	849.60 ± 14.31^{b} (00.00%)
Positive control	Diclofenac	252.80 ± 12.77^{b} (07.58%)	257.60 ± 81.05^{a} (69.87%)
DCM: Methanolic Leaf extracts	50 mg/kg	240.40 ± 11.37^{b} (12.39%)	743.20 ± 38.59^{b} (12.04%)
	100 mg/kg	180.00 ± 24.28^{a} (34.81%)	794.40 ± 25.96^{b} (06.04%)
	150 mg/kg	250.80 ± 09.78^{b} (24.10%)	804.00 ± 30.60^{b} (05.39%)

Values are expressed as Mean ± SEM for five animals per group. Statistical comparison were made within a column and values with the same superscript are not significantly different by ANOVA followed by Tukey's post hoc test ($p>0.05$). Figures in paranthesis indicate percent paw licking inhibition. Formalin=2.5%; DMSO=10%; Diclofenac=15 mg/kg.

Table 3: Antinociceptive effects of DCM: Methanolic leaf extract of *Maytenus obscura* (A Rich.) in Rats Model.

On the other hand, *Maytenus obscura* contained phenolics, trepenoids and saponins. However, alkaloids, flavanoids, steroids and cardiac glycosides were absent (Table 3).

Discussion

The present study was designed to evaluate the antinociceptive properties of the DCM: Methanolic leaf extracts of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A.Rich.). To evaluate antinociceptive properties of the extracts, formalin-induced paw licking test was used for its applicable, reliable and high specificity for antinociceptive responses [13,15]. The formalin test has been described as a convenient method for producing and quantifying pain in rats [16]. The test employs an adequate pain stimulus to which the animals show a spontaneous response and it is sensitive to commonly used analgesics. The pain stimulus, a continuous rather than a transient one, may have resemblance to some kinds of clinical pain and observations are made on animals which are restrained only lightly or not at all [11]. The advantage of the formalin model of nociception was that it could discriminate between central and peripheral pain components. The test consists of two different phases which could be separated in time: the first one occurs in the first 5 minutes after the formalin injection was generated in the periphery through the activation of nociceptive neurons by the direct action of formalin and the second phase occurring between the 15th and 30th minute after formalin injection, occurred through the activation of the ventral horn neurons at the spinal cord level [17]. Rats were subcutaneously administered with 0.01 ml of 2.5% formalin on the dorsal part of the rat hind paw. These definite shows whether the licking was genuinely due to formalin injected into the paw because

sometimes, the animals lick the forepaw under normal physiological condition.

Neurogenic pain is caused by the direct activation of nociceptive nerve terminals, whereas inflammatory pain is mediated by a combination of peripheral input and spinal cord sensitization. Formalin produces significant increases in spinal levels of different mediators related to both neurogenic such as amino acids, kinins and inflammatory pathways such as prostaglandins, leukotrienes [18]. During the early phase of the formalin test, chemoreceptors are activated on the peripheral terminals of primary afferents to evoke the release of proinflammatory peptides, producing neurogenic pain [19]. The late phase is either the result of sensitization of dorsal horn neurons (central sensitization), inflammation-induced hyperactivity of primary afferent nociceptors, or a combination of both [20]. During this phase glutamate is released [21] and glutamate receptor expression increases [22]. These changes produce pain behavior.

In this study, the DCM: Methanolic leaf extracts of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A.Rich.) showed a significant antinociceptive effect by reducing the formalin-induced licking time. The leaf extract *C. volkensii* reduced pain by between 6.82-15.24% only in the early phase while the leaf extracts of *M. obscura* reduced it by between 12.39-34.81% (in the early phase) and between 6.4-12.4% (in the late phase). Diclofenac reduced pain by between 7.58-9.66% (in the early phase) and by 69.87% in the late phase. This suggests both analgesic effects on the nociceptor blockage and an inhibition of the synthesis and/or release of inflammatory pain mediators such as PGs. The extracts, however, did not inhibit both phases equally. Hence, it is possible to suggest that the DCM: Methanolic leaf extracts of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A.Rich.) contain centrally and/or peripherally acting analgesic phytochemicals.

The antinociceptive effect of DCM: Methanolic leaf extracts of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A.Rich.) can be attributed to one or more groups of the phytoconstituents observed in the extracts. Several studies have shown the antinociceptive activity of such compounds. According to refs. [23,24] the antinociceptive effects of *T. foenum-graecum* and *V. tricolor* are attributed to the flavonoids present in the extracts. In addition, according to several studies, flavonoids are widely shown to inhibit both the cyclooxygenase and lipoxygenase pathways and also inhibiting the nitric oxide synthase [25]. Flavonoids are also shown to target prostaglandins which are involved in the pain perception through moderating opioidergic mechanism [26].

Conclusions

In conclusion, the present study has demonstrated the anti-inflammatory and antinociceptive potential of DCM: Methanolic leaf extract of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A.Rich.) in animal models. It is evident from the study that the antinociceptive activity of *Maytenus obscura* (A.Rich.) at 50 mg/kg body weight in early phase is almost similar to the standard diclofenac group. Therefore, the DCM: Methanolic leaf extract of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A.Rich.) might help in preventing pain complications and serve as good bio-resources for generating a readily available herb formulation that is more effective in the treatment of pain conditions, which is cheaper than the conventional synthetic drugs. This study scientifically confirms and supports the traditional use of *Caesalpinia volkensii* Harms and *Maytenus obscura* (A.Rich.) for management of pain condition.

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