The plants were collected from Siakago County, Embu County, Kenya. The fresh leaves of *Caesalpinia volkensii* and *Maytenus obscura* were identified and collected with the help of local traditional medicinal practitioners. 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. The animals were kept in the standard cages acquired and bred in the animal breeding and experimentation facility of the department of Biochemistry and Biotechnology, Kenyatta University for studies. The plant samples were provided to an acknowledged taxonomist for botanical authentication and a voucher specimen deposited at the Kenyatta University Herbarium.

**INTRODUCTION**

Inflammation is the response of living tissue to noxious stimuli, such as pathogens and irritant agents, which involves changes in blood flow, increased vascular permeability and leucocytes migration to the inflamed site [1]. Although a considerable number of anti-inflammatory drugs are available for the treatment of inflammation, there is a continuous search for new compounds as therapeutic alternatives, because these drugs exert a wide range of side effects and low efficacy, especially for chronic diseases [2]. Natural products have been one of the most successful sources for the discovery of new therapeutic agents to benefit those afflicted by inflammatory diseases [3] NSAIDs are amongst the most commonly used anti-inflammatory agents and they act to inhibit COX enzymes and reduce the formation of prostaglandins. NSAIDs can cause liver damage [4] renal failure [5], septic meningitis [6] and can interfere with bone fracture healing [7].

A study done in the Washambaa community of Tanzania reported twenty two plants as being routinely used by the community for the management of inflammation. Although a considerable number of anti-inflammatory drugs are available for the treatment of inflammation, there is a continuous search for new compounds as therapeutic alternatives, because these drugs exert a wide range of side effects and low efficacy, especially for chronic diseases [2]. Natural products have been one of the most successful sources for the discovery of new therapeutic agents to benefit those afflicted by inflammatory diseases [3] NSAIDs are amongst the most commonly used anti-inflammatory agents and they act to inhibit COX enzymes and reduce the formation of prostaglandins. NSAIDs can cause liver damage [4] renal failure [5], septic meningitis [6] and can interfere with bone fracture healing [7].

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**MATERIALS AND METHODS**

**Collection and preparation of plant materials**

The fresh leaves of *Caesalpinia volkensii* and *Maytenus obscura* were identified and collected with the help of local traditional medicinal practitioners. 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. The plant samples were provided to an acknowledged taxonomist for botanical authentication and a voucher specimen deposited at the Kenyatta University Herbarium.
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 anti-inflammatory activity

The animals were selected 24h prior to experimentation. The experimental animals were divided into six groups of five (n=5) and treated as shown in table 1.

Table 1: Treatment protocol for evaluation of anti-inflammatory activities

<table>
<thead>
<tr>
<th>Group</th>
<th>Status</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>Carrageenan only</td>
</tr>
<tr>
<td>II</td>
<td>Negative control</td>
<td>Carrageenan+10% DMSO</td>
</tr>
<tr>
<td>III</td>
<td>Positive control</td>
<td>Carrageenan+15 mg/kg diclofenac</td>
</tr>
<tr>
<td>IV</td>
<td>Experimental group A</td>
<td>Carrageenan+ 50 mg/kg+10% DMSO</td>
</tr>
<tr>
<td>V</td>
<td>Experimental group B</td>
<td>Carrageenan+ 100 mg/kg+10% DMSO</td>
</tr>
<tr>
<td>VI</td>
<td>Experimental group C</td>
<td>Carrageenan+ 150 mg/kg+10% DMSO</td>
</tr>
</tbody>
</table>

Carrageenan = 100 µg DMSO = 10%

The paw edema/Inflammation was induced by carrageenan (0.01 ml, 1% w/v in normal saline) into sub-plantar tissue of right hind paw of mice. The linear paw circumference was measured at hourly interval during the daytime in a quiet laboratory setting with ambient illumination and temperature similar to those of the animal house. During the study, all the tests were carried out on animals selected 24h prior to experimentation. The experimental data on the increase in the diameter of the paw were 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 groups was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test 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 extract of Caesalpinia volkensii and leaf extracts of Maytenus obscura. The value of P ≤ 0.05 was considered significant. Analysis of the data was done using Minitab statistical software.

Table 2: Effects of DCM: methanolic leaf extracts of Caesalpinia volkensii Harms on carrageenan induced inflammation in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Percent change in paw circumference after drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0h</td>
</tr>
<tr>
<td>Baseline</td>
<td>None</td>
<td>100±0.00</td>
</tr>
<tr>
<td>Negative control</td>
<td>Carrageenan+DMSO</td>
<td>100±0.00</td>
</tr>
<tr>
<td>Positive control</td>
<td>Carrageenan+Diclofenac+DMSO</td>
<td>100±0.00</td>
</tr>
<tr>
<td>DCM: methanolic</td>
<td>Carrageenan+50 mg/kg</td>
<td>100±0.00</td>
</tr>
<tr>
<td>leaf extract</td>
<td>Carrageenan+100 mg/kg</td>
<td>100±0.00</td>
</tr>
<tr>
<td></td>
<td>Carrageenan+150 mg/kg</td>
<td>100±0.00</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SEM for five animals per group. Statistical comparison was 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). Carrageenan = 100 µg; DMSO = 10% Diclofenac = 15 mg/kg.

RESULTS

Anti-inflammatory effects of DCM: Methanolic extract of Caesalpinia volkensii Harms on carrageenan induced edema in mice.

The three dose levels (50, 100 and 150 mg/kg body weight) of the DCM: Methanolic leaf extracts of C. volkensii reduced carrageenan induced inflammation in mice. This was indicated by the reduction of the hind paw circumference.

In the first hour, the DCM: Methanolic leaf extracts of C. volkensii at all the doses levels (50, 100 and 150 mg/kg body weight) reduced the paw circumference by 6.5%, 6.19% and 10.99%, respectively (fig. 1). In this hour, the anti-inflammatory activity of the DCM: Methanolic leaf extracts of C. volkensii at all the three doses levels (50, 100 and 150 mg/kg body weight) was not significantly different compared to the positive control groups (p>0.05; table 2). However, the reduction of paw circumference by DCM: Methanolic leaf extracts of C. volkensii at the dose level of 50 and 100 mg/kg body weight and diclofenac was not significantly different compared to baseline (p>0.05; table 2).

In the second hour, the DCM: Methanolic leaf extracts of C. volkensii at all the doses levels reduced the paw circumference by 9.1%, 7.41% and 10.99%, respectively (fig. 1). In this hour, the anti-inflammatory activities of DCM: Methanolic leaf extract of C. volkensii at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) were significantly different compared to baseline and negative control groups (p<0.05; table 2). Besides, in this hour, the anti-inflammatory activity of extracts at all the dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) was comparable to the standard drug (diclofenac) (p>0.05; table 2).

In the third hour, the DCM: Methanolic leaf extracts of C. volkensii at the dose levels of 50, 100 and 150 mg/kg body weight reduced in paw circumference of mice to be 87.00%, 88.88% and 86.62%, respectively (table 2). At this hour, the group of mice treated with 150 mg/kg of the herbal extract exhibited the highest anti-inflammatory activity.
inflammatory effect (fig. 1; table 2). Although anti-inflammatory activities of DCM: Methanolic leaf extracts of C. volkensii at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) was not significantly different compared to baseline (p>0.05; table 2), they were comparable to diclofenac (reference drug) (p>0.05; table 2). In addition, the anti-inflammatory activities of the extracts were significantly different compared to the negative control group (p<0.05; table 2).

Effects of DCM: Methanolic leaf extracts of M. obscura on carrageenan induced inflammation in mice

In general, the DCM: Methanolic leaf extract of Maytenus obscura (A. Rich.), at all the three dose levels (50, 100 and 150 mg/kg body weight), inhibited carrageenan induced inflammation in mice demonstrated by the reduction in the paw circumference.

In the first hour of the test period, the DCM: Methanolic leaf extract of M. obscura at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) reduced paw circumference to 93.11%, 88.96% and 95.15%, respectively (table 3). In this hour, although the anti-inflammatory activities of DCM: methanolic leaf extracts of C. volkensii at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) was not significantly different compared to baseline (p>0.05; table 3), they were comparable to diclofenac (reference drug) (p>0.05; table 3). However, the anti-inflammatory activities of the extracts were significantly different compared to the negative control group (p<0.05; table 3).

In the second hour, the percent paw circumference reduction by the DCM: Methanolic leaf extracts of M. obscura at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) were 11.77%, 18.52% and 11.12%, respectively (fig. 2). At this hour, the group of mice treated with 100 mg/kg of the herbal extract exhibited the highest anti-inflammatory effect (fig. 2; table 3). The DCM: methanolic leaf extracts of M. obscura at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) significantly reduced the carrageenan-induced paw edema compared to the baseline and negative control groups (p<0.05; Table 3) but were comparable to diclofenac (p>0.05; Table 3).

In the third hour, the DCM: methanolic leaf extracts of M. obscura at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) reduced the paw circumference to 77.89%, 80.11% and 87.63%, respectively (table 3). In this hour, the anti-inflammatory effects of the DCM: Methanolic leaf extract of M. obscura at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) were significantly different from the baseline and negative control groups (p<0.05; table 3). The anti-inflammatory effects of the DCM: Methanolic leaf extracts of M. obscura at all the three dose levels (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) was comparable to the reference drug. However, the group of mice treated with 50 mg/kg was even better than the other dose levels as well as diclofenac for it reduced paw circumference by 22.36% while diclofenac reduced it by 10.47% (fig. 2).

Table 3: Effect of DCM: Methanolic leaf extracts of M. obscura on carrageenan induced inflammation in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Percent change in paw circumference after drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0h</td>
</tr>
<tr>
<td>Baseline</td>
<td>None</td>
<td>100±0.00</td>
</tr>
<tr>
<td>Negative control DMSO</td>
<td>100±0.00</td>
<td>112.57±5.14</td>
</tr>
<tr>
<td>Positive control Diclofenac</td>
<td>100±0.00</td>
<td>91.89±1.37</td>
</tr>
<tr>
<td>DCM: methanolic leaf extract</td>
<td>50 mg/kg</td>
<td>100±0.00</td>
</tr>
<tr>
<td></td>
<td>100 mg/kg</td>
<td>100±0.00</td>
</tr>
<tr>
<td></td>
<td>150 mg/kg</td>
<td>100±0.00</td>
</tr>
</tbody>
</table>

All 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). Carrageenan = 100 µg DMSO = 10%; Diclofenac = 15 mg/kg.

Fig. 2: Effects of Maytenus obscura (A. Rich.) on the percent change in carrageenan induced inflammation in mice

In comparison, the DCM: Methanolic leaf extract of Caesalpinia volkensii Harms exhibited more anti-inflammatory effect than DCM: Methanolic leaf extract of M. obscura at all dose levels in the third hour of the test period. However, DCM: Methanolic leaf extract of Maytenus obscura (A. Rich.) at the dose level of 150 mg/kg body weight had the most effective anti-inflammatory effect than Caesalpinia volkensii Harms in the first and second hours (fig. 3).

Phytochemical screening

As table 4 shows, Caesalpinia volkensii contained flavonoids, steroids and phenolics whereas, alkaloids, terpenoids, saponins and cardiac glycosides were absent. On the other hand, Maytenus obscura contained phenolics, treponoids and saponins. However, alkaloids, flavonoids, steroids and cardiac glycosides were absent (table 4).
The significant inhibitory activity shown by the DCM: Methanolic extracts of Caesalpinia volkensii Harms and Maytenus obscura at all dose levels (50, 100, and 150 mg/kg body weight) over a period of 3h in carrageenan-induced inflammation was quite similar to that exhibited by the group treated with diclofenac. The highest percentage paw edema inhibition activity by DCM: Methanolic leaf extracts of Caesalpinia volkensii Harms was 13.42% at the dose level of 150 mg/kg body weight. Previous studies with some other plants like Plumeria acuminata [24] showed the same effect in this model. These results indicate that the extract acts in later phase in a dose dependent manner, probably involving arachidonic acid metabolites, which produce an edema dependent on neutrophil mobilization. This dose dependent anti-inflammatory effect in later phase could be explained by passive diffusion of active principles across the cell membrane in the peritoneal cavity.

Phytochemical screening done on the DCM: Methanolic leaf extract of Caesalpinia volkensii Harms indicated that it contains flavonoids, phenolics and steroids. The observed anti-inflammatory effect might be due to the presence of flavonoids. Flavonoids have been reported to inhibit prostaglandin synthesis [25]. It is ubiquitously known that flavonoids have a great potential as anti-inflammatory agents [26]. Therefore, it is postulated that flavonoids in the extract may correlate appropriately for the present activities.

The dose ranges used in this study were within the dose ranges used by [27], who used doses of 50, 100 and 150 mg/kg body weight while evaluating analgesic and anti-inflammatory studies of the methanolic extract of Anisopus monii (N. E. Br) in rodents. [28] also used levels of 50, 100 and 200 mg/kg body weight while evaluating the antinociceptive antipyretic effects of DCM: Methanolic leaf extracts of Solanum incanum (Linnaeus) in animals models. However, [29] while examining the anti-inflammatory activities of methanolic leaf extract of Mimusops elengi L. used dose levels of 100, 200 and 500 mg/kg body weight in rats.

The reduction in the paw circumference in case of treatment with Caesalpinia volkensii Harms and Maytenus obscura (A. Rich.) reflects that they are suitable anti-inflammatory agents comparable to the standard drug (diclofenac) at all dose levels. That the dose level of 150 mg/kg body weight of the DCM: Methanolic leaf extracts of Caesalpinia volkensii Harms was more effective than diclofenac suggests a possible better blockade of prostaglandins biosynthesis or mimicry of diclofenac action by the active principles in the extracts.

CONCLUSION
In conclusion, the present study has demonstrated the anti-inflammatory potential of DCM: Methanolic leaf extract of Caesalpinia volkensii Harms and Maytenus obscura (A. Rich.) in animal models. The significant reduction of paw circumference in mice when treated with standard drugs as well as different doses of extracts of Caesalpinia volkensii Harms and Maytenus obscura (A. Rich.) reflects that they are endowed with potent anti-inflammatory properties.

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 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 inflammatory conditions.

ACKNOWLEDGEMENT
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CONFLICT OF INTERESTS
Declared None

REFERENCES


