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(RESEARCH ARTICLE)

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Antitussive effects of *Musanga cecropioides* in animal models

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Abstract

This study investigates the antitussive effects of *Musanga cecropioides*, a traditional medicinal plant, using animal models. *Musanga cecropioides* extracts were prepared from the leaves, roots, and root sap, and their effects were evaluated on mucus expectoration and citric acid-induced cough in mice and guinea pigs, respectively. The results indicated that aqueous and methanol extracts of *Musanga cecropioides* leaves and roots significantly increased mucus secretion and reduced cough frequency, with the highest effectiveness observed in methanol root extract at 100 mg/kg. These findings suggest that *Musanga cecropioides* possesses promising antitussive properties, supporting its traditional use in treating cough and respiratory ailments. This natural remedy could offer a safer alternative to conventional cough suppressants, warranting further clinical studies to confirm its efficacy and safety in humans.

Keywords: Antitussive; Expectorant; *Musanga cecropioides;* Cough suppression; Traditional medicine

1. Introduction

Cough is the most common symptom of airway and lung disease [1]. Cough may be the most prominent symptom complained of by patients with chronic respiratory disease such as asthma [2]. The effect of persistent cough itself may be harmful and deleterious to the patient by interfering with breathing, social activities and sleep, and by causing deterioration in the quality of life, social embarrassment, urinary incontinence, muscle ache, insomnia, fatigue, chest and thorax pain [3]. Available drugs for the management of cough include expectorants, antitussives and glucocorticoids most of which are synthetic drugs, and are associated with side effects [4].

In the United States of America, a national medical case survey reported that in 1991 cough was the most common complaint for which patients sought medical attention, and the second most common reason for a general medical consultation [5]. Treatment of the causes of cough may include anti-inflammatory approaches such as the cough associated with eosinophilic inflammation in the airways as in asthma, cough-variant asthma or eosinophil bronchitis [6]. Partly because there is no apparent cause associated with a persistent cough in many patients, it is clear that the development of effective antitussives (i.e. drugs that specifically block cough whatever the cause) is needed. Currently, the most commonly used antitussives are the centrally acting opiates (acute relievers of cough) such as codeine, dihydrocodeine or pholcodeine. Sometimes for the intractable cough in terminal disease, morphine or diamorphine is used, with the advantage that these also possess analgesic properties. However, at their effective doses, these antitussives are associated with side effects such as drowsiness, nausea, vomiting, constipation, and often cause physical dependence. Development of new antitussives has occurred on a number of fronts including in phytomedicine [7].

Musanga cecropioides is an important medicinal plant as most of its parts are used in traditional medicine in Nigeria, Cameroun and other countries in Africa [8]. The leaves, stem bark, root bark and root sap are used to manage cough, pulmonary complaints and in the treatment of upper respiratory tract infection [9, 10, 11, 12, 13, 14, 15]. The aim of

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this study was to investigate the antitussive and expectorant properties of *Musanga cecropioides* leaves, root and root sap, in order to ascertain ethnomedicinal reports.

2. Materials and methods

2.1. Plant Sample Collection and Preparation

2.1.1. Plant Sample Collection

Fresh leaves, root and root sap of *Musanga cecropioides* was collected from a private farmland in Ulorin village, Ovia North Local Government Area of Edo State, Nigeria in April 2017. A young branch bearing leaves, flowers and fruit was harvested for identification and authenticated by Prof. MacDonald Idu of the Department of Plant Biology and Biotechnology, University of Benin, Benin City. The voucher specimen was deposited in the same Department and voucher specimen number obtained (UBH-M500). The leaf and root samples needed for this work were washed to get rid of debris, then chopped into smaller bits and dried in an aseptic environment for a period of one and two weeks for the leaves and root respectively. The dried plant materials were then pulverized to powdered form using the British Milling Machine by Gallehamp. The powdered plant materials were then stored in air tight containers awaiting further use as explained in the different sections that follows.

The sap was however collected overnight by making an incision on the root and placing a collection bowl underneath for the fluid to flow. The sap was stored in fitly covered containers and refrigerated at 4^{o}C prior to use.

2.1.2. Plant Extract Preparation

A total of 200 g of sample (powder) was diluted in 1200 ml of solvent (distilled water, methanol) in a glass jar (3000 ml). The mixture was allowed to settle for 72 hours. The mixtures were filtered and the resulting filtrate was concentrated using a water bath as described by Oshomoh et al. [16].

2.2. Antitussive Study

2.2.1. Experimental Animals

Antitussive experiment was performed using 48 adult guinea pigs of either sex weighing 260 – 460 g. Mucus expectorant activity was evaluated using 90 mice of either sex weighing 17 – 33 g. The guinea pigs were obtained from the animal facility of the Department of Physiology, Ambrose Alli University, Ekpoma, Nigeria. The mice were obtained from a private animal farm in Ibadan, Oyo State, Nigeria. All animals were allowed two weeks acclimatization in the animal facility of the Faculty of Science, Edo State University Uzairue, Iyamho, Edo State. They were all allowed free access to pellets and tap water and were exposed to natural light-dark cycle and room temperature. All animals were handled according to standard protocols for the use of laboratory animals [17] and the experiments were overseen by members of Ethics Committee of the Faculty of Science, Edo State University Uzairue.

2.2.2. Mucus Expectoration (Phenol Red) Experiment

This was based on the method first described by Engler and Szelenyi [18] with slight modifications by Ozolua et al. [19]. The animals for this experiment were grouped (n=5) as follows: (1) Control group given 1 ml/kg distilled water; (2) 15 mg/kg Bromohexine; (3) 50 mg/kg Sodium cromoglycate; (4) 50 mg/kg *Musanga cecropioides* aqueous leaf extract; (5) 100 mg/kg *Musanga cecropioides* aqueous leaf extract; (6) 200 mg/kg *Musanga cecropioides* aqueous leaf extract; (7) 50 mg/kg *Musanga cecropioides* methanol leaf extract; (8) 100 mg/kg *Musanga cecropioides* methanol leaf extract; (9) 200 mg/kg *Musanga cecropioides* methanol leaf extract; (10) 50 mg/kg *Musanga cecropioides* aqueous root extract; (11) 100 mg/kg *Musanga cecropioides* aqueous root extract; (12) 200 mg/kg *Musanga cecropioides* aqueous root extract; (13) 50 mg/kg *Musanga cecropioides* methanol root extract; (14) 100 mg/kg *Musanga cecropioides* methanol root extract; (15) 200 mg/kg *Musanga cecropioides* methanol root extract; (16) 0.5 ml/kg *Musanga cecropioides* crude root sap; (17) 1 ml/kg *Musanga cecropioides* crude root sap; (18) 2 ml/kg *Musanga cecropioides* crude root sap.

All treatments were administered orally except for sodium cromoglycate that was administered intraperitoneally (i.p.).

On the 8th day, after an overnight fast, treatment was done as usual and the animals in the third group were administered 50 mg/kg sodium cromoglycate. About 30 minutes later the secretagogue, ammonium chloride was administered to all groups (5 mg/kg orally). Thirty minutes after the administration of ammonium chloride, each mouse was injected with phenol red (500 mg/kg i.p.). All the mice were sacrificed by cervical dislocation 30 min after phenol

red injection. The 2 cm of trachea was removed from the thyroid cartilage to the main stem bronchi. Each trachea was kept for 30 min in 2 ml normal saline. Sodium hydroxide (0.1 ml, 1 M) was added to the fluid to stabilize the pH. The absorbance of phenol red released from the trachea was read at 460 nm using a T80+UV/VIS spectrophotometer (PG Instruments Ltd, Beijing, China). A standard curve (graph of absorbance against concentration, R2 = 0.947) was plotted from which the concentrations of phenol red were extrapolated.

2.2.3. Antitussive Screening Protocol

This was based on the guinea pig cough model described by Nadig and Laxmi [20].

A day before the experiment, guinea pigs were placed individually in a glass chamber (60 × 36 × 60 cm) for 5 min before cough was induced by exposure to citric acid aerosol (7.5% w/v) using an Omron® (Omron Health Care Ltd, Japan) compressor nebulizer (rate of 0.4 ml/min and particle size 5 µm) for 5 min. The animals exhibiting at least 10 bouts of cough were selected for the study and fasted overnight but with access to water. The selected animals were randomly allotted to experimental groups (n=4 per group). The animals were treated orally as outlined below:

(1) Control group given 1 ml/ kg distilled; (2) 25 mg/kg dihydrocodeine; (3) 50 mg/kg *Musanga cecropioides* aqueous leaf extract; (4) 100 mg/kg *Musanga cecropioides* aqueous leaf extract; (5) 50 mg/kg *Musanga cecropioides* methanol leaf extract; (6) 100 mg/kg methanol leaf extract; (7) 50 mg/kg *Musanga cecropioides* aqueous root extract; (8) 100 mg/kg *Musanga cecropioides* aqueous root extract; (9) 50 mg/kg *Musanga cecropioides* methanol root extract; (10) 100 mg/kg *Musanga cecropioides* methanol root extract; (11) 0.5 ml/kg *Musanga cecropioides* crude root sap; (12) 1 ml/kg *Musanga cecropioides* crude root sap.

An hour after administration, they were re-exposed to citric acid aerosol (as earlier described) and the numbers of cough bouts were recorded.

Percentage suppression of cough was calculated using the formula:

$$\frac{\text{Number of Pre Treatment Cough Bouts} - \text{Number of Post Treatment Cough Bouts}}{\text{Number of Pre Treatment Cough Bouts}} \times 100$$

2.3. Data Analysis

Data were subjected to one way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons using Graphpad prism software (version 6.01). Results are expressed as mean ± SEM and values of $P < 0.05$ were considered as statistically significant.

3. Results

3.1. Mucus Expectoration (Phenol Red) Experiment

Figures 1, 2, 3, 4 and 5 show the results on the effects of *Musanga cecropioides* aqueous leaf extract, methanol leaf extract, aqueous root extract, methanol root extract and crude root sap respectively, on mucus expectoration in mice using phenol red dye as an indicator.

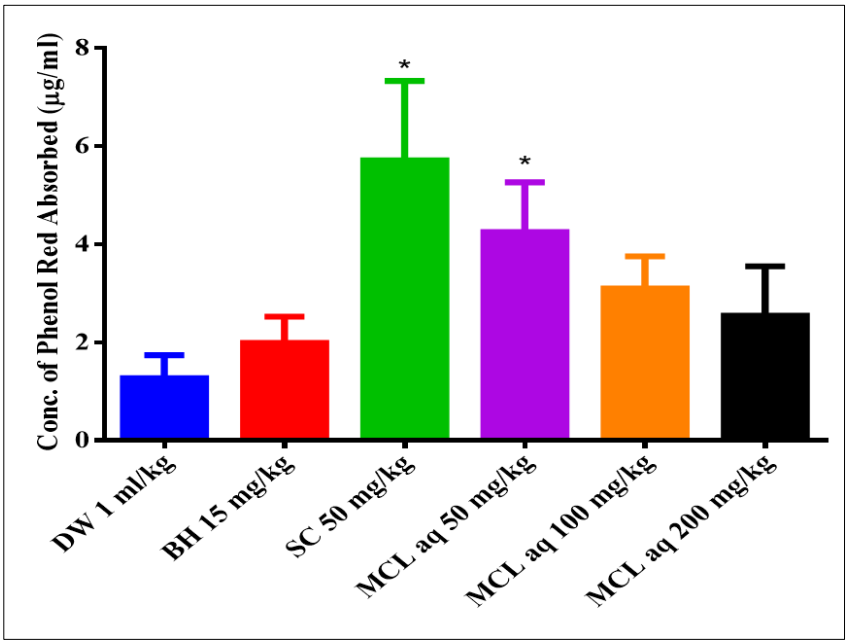
In Figure 1 *Musanga cecropioides* aqueous leaf extract at 50 mg/kg significantly increased ($P < 0.05$) the phenol red dye concentration when compared to the control group treated with only distilled water, however at the higher concentrations administered (100 and 200 mg/kg), concentration of phenol red was reduced but not significantly different from the control.

In Figure 2 *Musanga cecropioides* methanol leaf extract at 100 mg/kg significantly increased ($P < 0.05$) the concentration of phenol red.

In Figure 3 *Musanga cecropioides* aqueous root extract at 100 mg/kg significantly increased ($P < 0.05$) the concentration of phenol red.

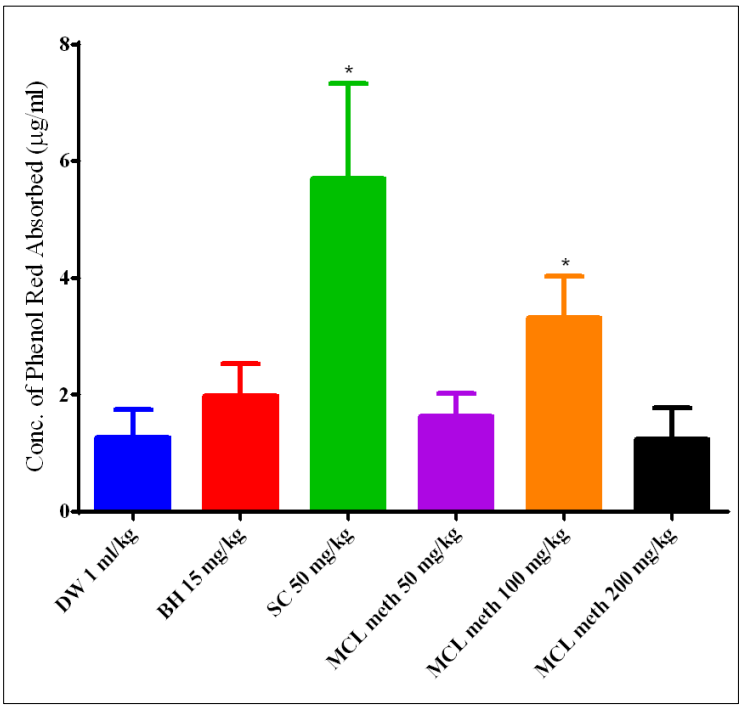
In Figure 4 *Musanga cecropioides* methanol root extract did not significantly alter the phenol red dye concentration when compared to the control group treated with only distilled water.

In Figure 5 *Musanga cecropioides* crude root sap did not significantly alter the phenol red dye concentration when compared to the control group treated with only distilled water.



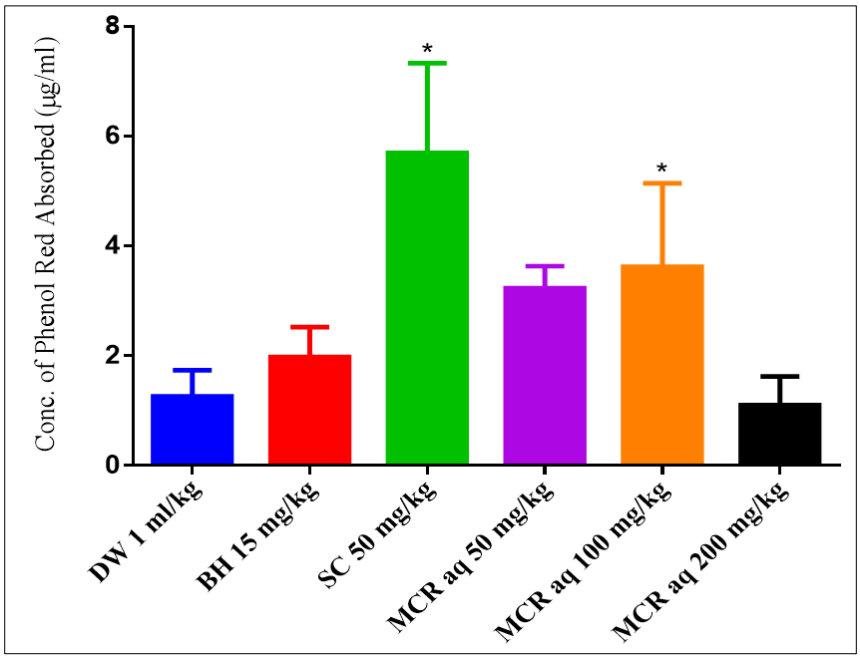
DW: distilled water; BH: Bromohexine hydrochloride; SC: Sodium cromoglycate; MCL: *Musanga cecropioides* leaves; AQ: aqueous; n=5; * significant values at P < 0.05, when compared to control group treated with only distilled water

Figure 1 Effect of *Musanga cecropioides* aqueous leaf extract on phenol red dye secretion in mice trachea



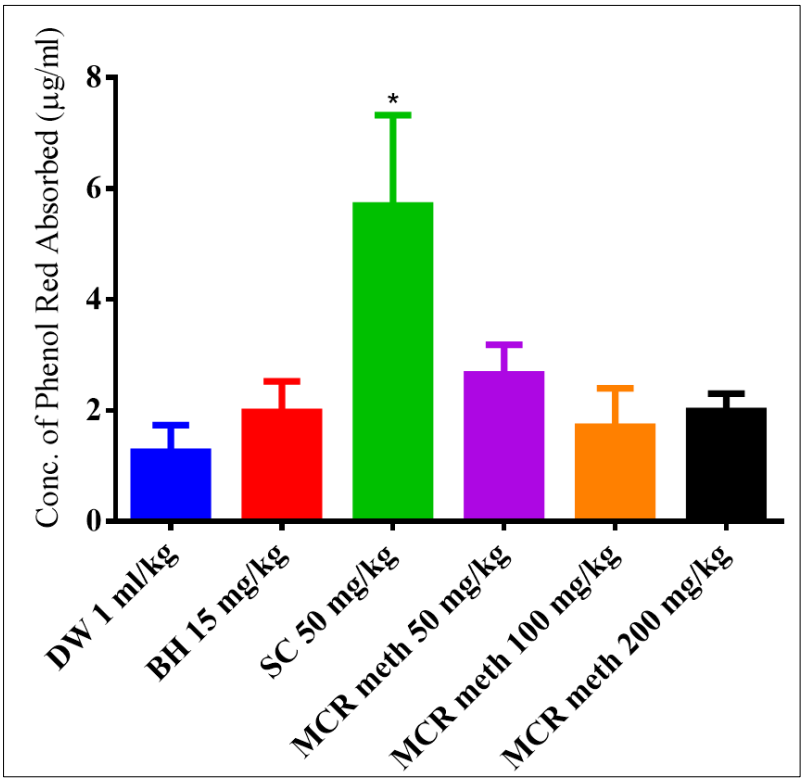
DW: distilled water; BH: Bromhexine hydrochloride; SC: Sodium cromoglycate; MCL: *Musanga cecropioides* leaves; METH: Methanol; n=5; * significant values at P < 0.05, when compared to control group treated with only distilled water

Figure 2 Effect of *Musanga cecropioides* methanol leaf extract on phenol red secretion in mice trachea



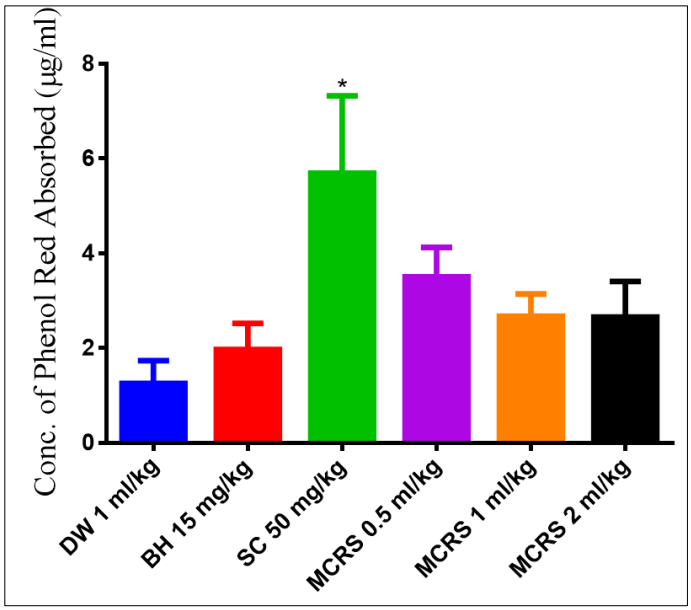
DW: distilled water; BH: Bromhexine hydrochloride; SC: Sodium cromoglycate; MCR: *Musanga cecropioides* root; AQ: aqueous; n=5; * significant values at P < 0.05, when compared to control group treated with only distilled water

Figure 3 Effect of *Musanga cecropioides* aqueous root extract on phenol red secretion in mice trachea



DW: distilled water; BH: Bromhexine hydrochloride; SC: Sodium cromoglycate; MCR: *Musanga cecropioides* root; METH: Methanol; n=5; * significant values at P < 0.05, when compared to control group treated with only distilled water

Figure 4 Effect of *Musanga cecropioides* methanol root extract on phenol red secretion in mice trachea

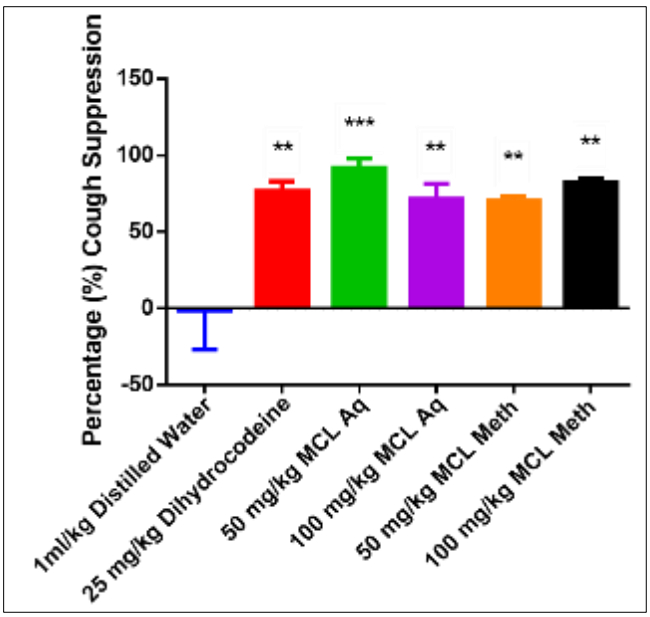


DW: distilled water; BH: Bromhexine hydrochloride; SC: Sodium cromoglycate; MCR: *Musanga cecropioides* root sap; n=5; * significant values at P < 0.05, when compared to control group treated with only distilled water

Figure 5 Effect of *Musanga cecropioides* crude root sap on phenol red secretion in mice trachea

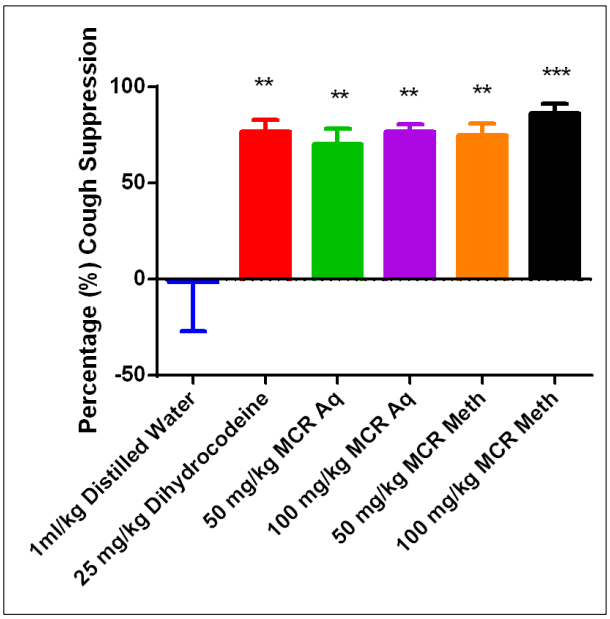
3.2. Antitussive Experiment (Citric acid-induced cough)

Figures 6, 7 and 8 shows the effect of *Musanga cecropioides* leaf extracts, root extracts and crude root sap respectively, on citric acid induced cough in guinea pigs. All samples significantly inhibited cough bouts (at P < 0.05, P < 0.01 and P < 0.001) when compared to the control group treated with 1 ml/kg distilled water. The antitussive activities observed in Figures 6, 7 and 8 were comparable to the control group treated with dihydrocodeine 25 mg/kg (76.81 ± 6.20%) however, *Musanga cecropioides* aqueous leaf extract at 50 mg/kg (figure 6) and methanol root extract at 100 mg/kg (figure 7) showed the best percentage inhibition of cough at 91.97 ± 5.90% and 86.50 ± 4.80% respectively. The lowest percentage inhibition was observed in the groups treated with *Musanga cecropioides* crude root sap (figure 8) at 0.5 ml/kg and 1 ml/kg, showing 62.98 ± 8.89% and 64.50 ± 6.88% respectively.



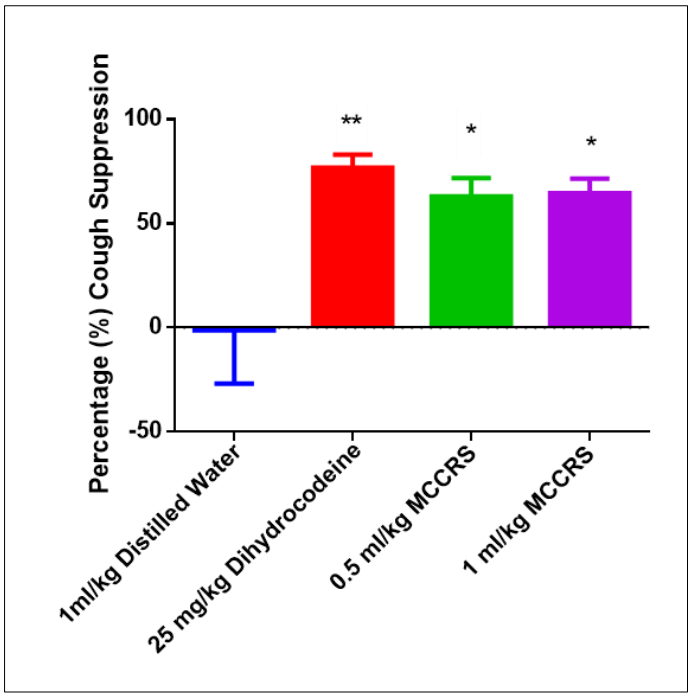
** significant values at P < 0.01; *** significant values at P < 0.001 when groups compared to control groups. AQ: aqueous; METH: Methanol. n=4 per group.

Figure 6 Antitussive effect of *Musanga cecropioides* leaf aqueous and methanol extracts on citric acid-induced cough in guinea pigs



** significant values at P < 0.01; *** significant values at P < 0.001 when groups compared to control groups. AQ: aqueous; METH: Methanol. n=4 per group.

Figure 7 Antitussive effect of *Musanga cecropioides* root aqueous and methanol extracts on citric acid-induced cough in guinea pigs



* significant values at P < 0.05; ** significant values at P < 0.01 when groups compared to control groups. MCCRS: *Musanga cecropioides* crude root sap. n=4 per group.

Figure 8 Antitussive effect of *Musanga cecropioides* crude root sap on citric acid-induced cough in guinea pigs

4. Discussion

Expectorants are known to increase the secretion and ease of elimination of sputum and phlegm in the respiratory tract through increased ciliary movement [21]. Hyper secretion of mucus in the airway is a major cause of cough and phlegm build-up, and this is associated with the development of chronic airway inflammation and altered lung function [22]. In

the phenol red experiment, *Musanga cecropioides* leaves, root and root sap exhibited expectorant effects. The aqueous leaf extract at 50 mg/kg, methanol leaf extract at 100 mg/kg and aqueous root extract at 100 mg/kg significantly increased mucus expectoration in the experimental animals. However, a dose dependent reduction in the concentration of phenol red was observed in the group administered aqueous leaf extracts. The observed expectorant effects in this study are comparable to previous reports [19, 23] and is attributable to the presence of flavonoids and the anti-inflammatory properties of *Musanga cecropioides* as earlier reported in literature [23, 24].

The citric acid induced cough in guinea pigs is one of the easily observable and accurate model of study the antitussive effects of drugs [25]. *Musanga cecropioides* leaf, root and root sap at all administered doses, significantly reduced the number of cough bouts in guinea pigs exposed to citric acid aerosol. The highest percentage reduction was observed in the group treated with the methanol root extract at 100 mg/kg. This was correlated with the reduced phenol red output in the expectorant study and in line with the reports of Ozolua et al. [19]. The antitussive properties of plants have been linked to the presence of flavonoids and terpenoids [23].

Musanga cecropioides extracts demonstrates promising antitussive effects in animal models, supporting its traditional use in treating coughs. This natural remedy could serve as a potential alternative to conventional cough suppressants, offering a safer profile with fewer side effects. Future research should focus on clinical trials to confirm its efficacy and safety in humans.

Compliance with ethical standards

Acknowledgement

Our gratitude goes to the Management of Edo State University Uzairue and for creating an enabling environment for the research to be carried out.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

All animal experiments were conducted in accordance with the ethical guidelines and regulations for animal care and use established by Edo State University Uzairue, Faculty of Science Ethical Committee. The study protocol was reviewed and approved under the approval number EDSU-BIOEC-00201. All efforts were made to minimize animal suffering and to reduce the number of animals used.

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