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THE ANTISPASMODIC EFFECT OF *Clerodendrum quadriloculare* (Blanco) Merr. LEAF EXTRACTS

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ABSTRACT

Clerodendrum quadriloculare, locally named *bagawak morado* decoction of leaves is used to relieve abdominal pain in folklore. However, scientific data on the biological activities of the plant are limited, thus prompting this research to provide in part the scientific basis for the plant's reputed use for smooth muscle relaxant effects. The methanolic extract of *Clerodendrum quadrilocualre* was subjected to phytochemical analysis, toxicity test and smooth muscle modulating effects using isolated rat ileum. Phytochemical analysis by thin layer chromatography revealed presence of flavonoids, steroids, triterpenes, coumarins, tannins, phenolics and alkaloids. Toxicity study showed that *C. quadriloculare* is non-toxic at 5000 mg/kg. Organ bath studies of the isolated rat ileum showed that DCM fraction comparable relaxation activity of the acetylcholine-induced contraction (EC_{50}=1376 µg/mL ± 0.03) as with the standard antagonist, Atropine ($p > 0.05$). The study indicates the potential use of *Clerodendrum quadriloculare* as a safe and effective treatment for smooth muscle spasm.

Keywords: *Clerodendrum quadriloculare*, spasm, Acetylcholine, Isolated ileum, Organ bath

INTRODUCTION

Clerodendrum quadriloculare (Blanco) Merr. is known as *bagawak morado* in Filipino. It is an endemic plant in the Philippines and is currently used as alternative medicine for indigestion and stomach pain[1]. Five or more leaves are boiled in water; decoction is taken half a cup twice daily. The bark from the plant is juiced and taken for stomach ache and gas pains. Despite the wide folkloric use of *C. quadriloculare*, there is no establish scientific studies on the pharmacologic activities of the plant. Toxicity profile of the plant has also not been established. Although there is lack of pharmacologic study on *C. quadriloculare*, sterol metabolites have been isolated by Macabeo, *et al* in 2008. Isolated sterols were identified as: 22-dehydroclоresterol, clerosterol, and 22-dehydroclerosterol 3β-O-β-D-(6'-o-margaroyl)-glucopyranoside[2]. These sterols were also isolated from the members of the Verbanaceae family where the plant was previously classified before it was transferred into Lamiaceae[3]. Isolation of the flavonoid hispidulin from *C. petasites*[6] was reported to cause relaxation of the tracheal smooth muscle contracted by the exposure to histamine. This suggests that *Clerodendrum* species may have smooth muscle relaxant effect. The present study aimed to evaluate the claimed therapeutic effect, i.e. antispasmodic, property of *C. quadriloculare* on isolated ileum from Sprague dawley rat. This will provide a scientific basis on the folkloric use of the plant.

MATERIALS AND METHODS

Plant Collection: The leaves of the *Clerodendrum quadriloculare* were collected in Zaragoza, Nueva Ecija, Philippines. The plant was identified by the curator of the UST Herbarium, Botany Laboratory, Research Center for the Natural Sciences (University of Santo Tomas) with the voucher specimen USTH4899.

Preparation of Extract: Leaves were air dried for approximately two weeks and powdered using the Wiley mill. The powdered material was subjected to exhaustive percolation using 95% methanol (1:10 w/v) for 24 hours. Percolation was repeated until the percolate was seen to be light green in color. Resulting percolate was concentrated under a reduced pressure at 40ºC until it produced a viscous dark brown extract (11% yield). Half of the collected crude extract was partitioned using the solvent-solvent partition method of increasing polarity namely: n-hexane, DCM, butanol and water fractions. All fractions obtained were concentrated *in vacuo*. The extracts obtained were subjected to phytochemical analysis.

Animals: Female ICR mice (25-35 g), and male Sprague dawley rats (350-450 g) were purchased from the Food and Drug Administration and were housed in the Thomas Aquinas Research Complex (TARC) Animal House having a controlled temperature 24±2ºC; relative humidity 75 ± 5% in a 12 hour light-dark cycle. Rodent chow and distilled water were given *ad libitum*. The animals were acclimatized for at least 5 days before the start of experiments. The experimental protocol was approved the Institute of Animal Care and Use Committee of the University of Santo Tomas (UST-IACUC) while Animal Research Permit was secured from the Department of Agriculture, Bureau of Animal Industry (Reference No. AR-2-14-128).

Drugs and chemicals: All drugs used in the experiment were purchased from Sigma-Aldrich, Singapore while all other chemicals used for the study were analytical grade obtained from Belman Incorporada, Philippines. Acetylcholine was diluted with phosphate buffer 7.4 to a concentration of $5x10^{-3}$ M, Atropine is diluted with ultrapure water and prepared into a concentration of $5x10^{-3}$ M.

Phytochemical Screening: Phytochemical screening was done through Thin Layer Chromatography (TLC). Crude methanolic extracts and its fractions were spotted on the silica gel plates. The plates were developed in the equilibrated chamber containing the most suitable solvent system. The developed chromatogram was visualized by inspecting under the UV light. Different spray reagents[7] were used to screen the different phytochemicals present in the plant extract and fractions.

Acute Oral Toxicity Test: Acute oral toxicity test was performed following the OECD Guideline 423 of 2001[4]. Healthy, nulliparous and non-pregnant mice were selected randomly (n=3). Food, but not water was withheld for 4 hours prior to induction of CQME. . The test substance (CQME in 10% Tween 80) was administered in a single dose using a gastric gavage designed for mice. The starting dose level is 300 mg/kg body weight and mortality is observed for 3days. If mortality was observed in 2/3 or 3/3 animals, the 300 mg/kg dose is considered toxic dose. However if 1/3 or 0/3 was observed then same dose was repeated again to confirm the toxic effect. If mortality was not observed, higher dose of 2000 mg/kg shall be administered and monitored in the same manner. If mortality was still absent for 2000 mg/kg, the use of 5000 mg/kg dose will be administered into three mice. Daily observations were noted until the 14th day of the experiment.

Anti-spasmodic studies: The same protocol was reviewed and approved by the UST-IACUC before the start of experiment. Male Sprague dawley rat weighing 450 g was used. The animal was sacrificed by cervical dislocation. The abdomen was opened and the proximal ileum was removed, washed and placed in Krebs-Henseleit solution. Segments of 1 cm were placed in 25 mL organ bath with a resting tension of 1 gram (preload). One end was attached to the force transducer (UGO Basile) that is connected to the bridge amplifier of the ADI PowerLab for the recording of the contraction changes[5]. Krebs-Henseleit solution[5] was composed of (mM): NaCl 118.1, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 5.6. The solution was freshly prepared and kept at 37ºC, pH 7.4 and aerated with carbogen (95% O_2/ 5% CO_2 mixture). The isolated ileum was allowed to equilibrate for 60 minutes with washings every 15 minutes. Precontraction with acetylcholine was done in four minutes followed by three times of buffer overflow. Recordings of the PowerLab were noted for the cumulative concentration response curves using the agonist acetylcholine (Ach), Atropine, and the extracts and fractions of *Clerodendrum quadriloculare* on the isotonic organ bath. The effect was allowed to reach a steady state at each concentration.

Statistics: After fitting the concentration-response curves, the EC_{50} was calculated using software. The

results were expressed as mean ± SEM of a given number of trials. For statistical evaluation multiple comparisons of the means were carried out by one-way analysis of variance followed by a post hoc test.

RESULTS

Phytochemical Screening: Results of the phytochemical screening done through TLC revealed the presence of the phytochemicals anthraquinones, alkaloids, coumarins, flavonoids, phenols, steroids, sterols, and tannins. Indoles, cardenolides and sugars were not present in the leaves of *Clerodendrum quadriloculare*.

Acute Oral Toxicity Test: Results showed that doses of 300 mg/kg to 5000 mg/kg body weight, no lethality and no signs of toxicity were observed during the period of 30 minutes, 4 hours, 24 hours, and 14 days on the treated female ICR mice. The study found out that a single administration of *Clerodendrum quadriloculare* leaf extract through the oral route on the doses of 300 mg/kg, 2000 mg/kg and 5,000 mg/kg did not produce mortality or changes the behavioral patterns in mice compared to the control animal.

Anti-spasmodic studies: The activities of the isolated rat ileum were measured by the transducer connected to the PowerLab. Maximum contraction induced by Acetylcholine (91.5 µg/mL) was recorded. Cumulative amounts of Atropine (154.9 µg/mL) were added to provide 31.54% relaxation. Concentration response curves to the *Clerodendrum quadriloculare* leaf extract and fractions (n-hexane, DCM, n-butanol, water) in Ach-induced contraction of the isolated rat ileum was fitted and shown in Figure 1. Post hoc analysis showed that the relaxation made between concentrations is significant (p value $0.000 < 0.05\ \alpha$). Based on their mean relaxation effect, *Clerodendrum quadriloculare* leaf extracts is dose dependent. The higher the concentration is, the higher the % relaxation is observed.

The crude methanolic extract effectively reduced the Ach-induce contraction to 24.20% while 30.17% relaxation is brought by hexane, 32.99% by DCM, 32.10% by butanol and 31.32% by the water fraction. The DCM fraction can be considered to be the most bioactive fraction in terms of smooth muscle relaxation because it has the highest mean % relaxation response. One way analysis of variance presents that all samples have no significant difference in their % relaxation ($p > 0.05$). The values are the same in all the treatments. The activities of all extracts are comparable to the standard drug atropine which brought 31.54% relaxation (Table 1). EC_{50} value of the DCM fraction is computed from the Graph Pad Prism v.6 estimate is 1,376 µg/mL. This means that in a concentration of 1,376 µg/mL, 50% relaxation can be observed in the Ach induced contraction of isolated rat ileum.

CONCLUSION

Among the several traditional claims, the usefulness of *Clerodendrum quadriloculare* in stomach pain had been emphasized on the compilation of the ethnopharmacological uses of Philippine endemic plants[1]. Hence, it was considered that investigations for these medicinal properties may give scientific authentication to the traditional claims. Moreover, this plant has not been subjected to any systematic pharmacological screening. The results of the acute toxicity test indicated that the *C. quadriloculare* was fairly non-toxic. A significant relaxation was observed on the Ach-induced contraction of isolated rat ileum using organ bath studies. The relaxation effect of the ileum indicated the antispasmodic activity of this plant. The response of the DCM fraction was seen to be the highest and is comparable to the standard drug, atropine. This study further indicates the potential use of *Clerodendrum quadriloculare* as a safe and effective treatment for spasm.

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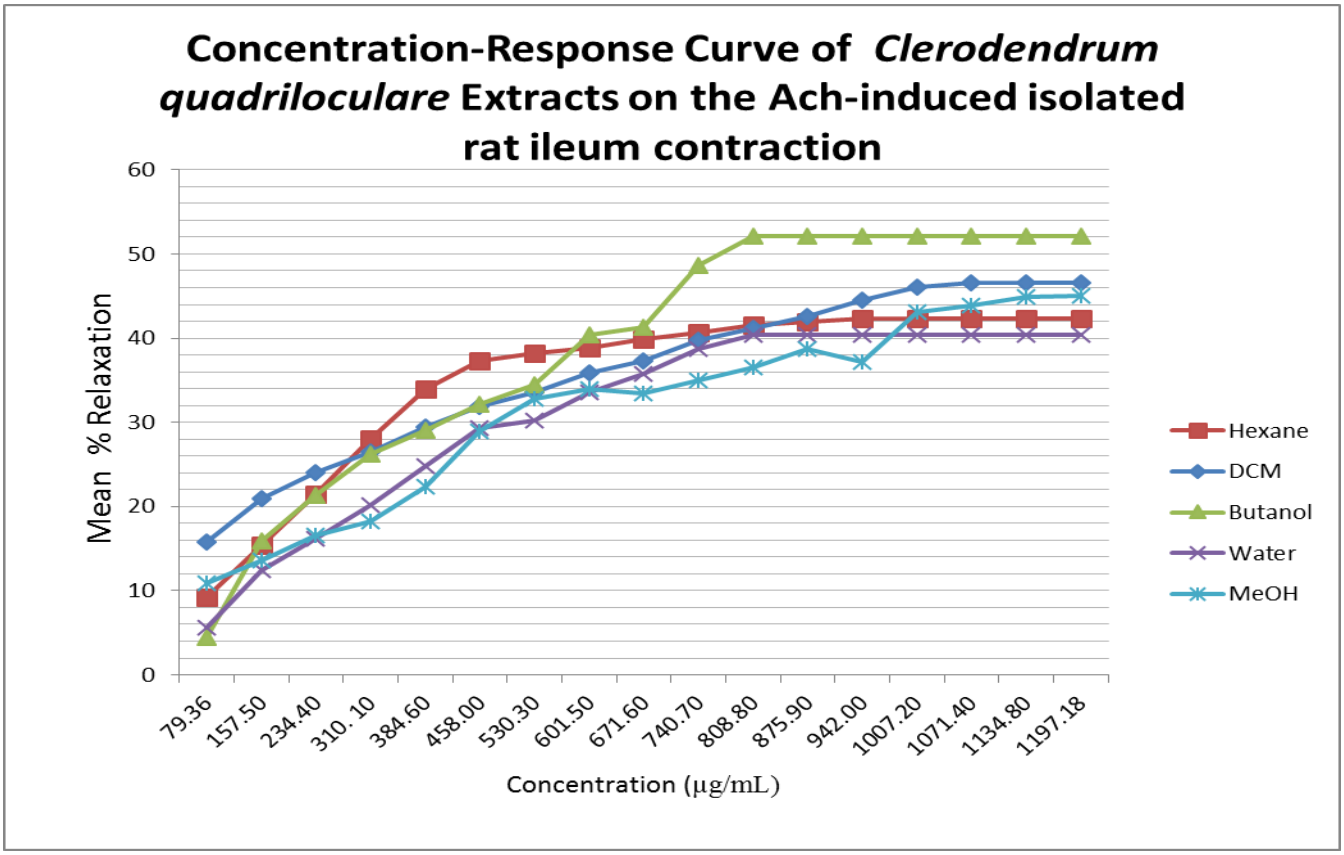

Figure 1 Concentration-response curve for the smooth muscle relaxant effect of *Clerodendrum quadriloculare* leaf extract and fractions n= 4 trials in the Ach-induced contraction of isolated rat ileum

Table 1: Mean relaxant % response of the extracts from *Clerodendrum quadriloculare* leaves

Antagonists	Concentration (organ bath) µg/mL	Mean relaxant % response ± SEM	EC_{50} (µg/mL) ± SEM
Acetylcholine	91.5	-48.61 ± 0.53	47.32 ± 0.00
Atropine	154.9	31.54 ± 0.01	1317 ± 0.00
Crude MeOH	1,134.8	24.20 ± 0.02	1599 ± 0.00
Hexane fraction	1,007.2	30.17 ± 2.77	1451 ± 0.21
DCM fraction	1,134.8	32.99 ± 0.39	1376 ± 0.03
Butanol fraction	942	32.10 ± 0.34	947.7 ± 0.00
Water fraction	1,007.2	31.32 ± 0.72	1249 ± 0.05

REFERENCES

1. Philippine Traditional Knowledge Digital Library on Health "Ethnopharm documentation for Bagawak." Prepared by Philippine Council for Health Research and Development, Philippine Institute of Traditional Alternative Healthcare and Institute of Herbal Medicine, National Institutes of Health, University of the Philippines Manila, http://www.tkdlph.com/index.php/ethnopharmacological-data/article/5928-bagawak
2. Macabeo A, Villafranca M., Aguinaldo A., Hussain H, Krohn K.. Clerosterols from *Clerodendrum quadriloculare*. Biochemical Systematics and Ecology, 2008, 36 (8): 659-660
3. Moldenke HN. Notes on the genus *Clerodendrum* (Verbanaceae) IV. Phytologia, 1985, 57: 334-365
4. Organisation for Economic Co-operation and Development (France); OECD Series on Testing and Assessment Number 24. Guidance Document on Acute Oral Toxicity Testing. Paris; 2001
5. Verspohl EJ, Fleer H, Antispasmodic activity of an extract from *Plantago lanceolata* L. and some isolated compounds. Phytomedicine, 2007, 14: 409-415
6. Hazekamp, A, Verpoorte, R, Panthong, A. Isolation of bronchodilator flavonoid from the Thai medicinal plant *Clerodendrum petsites*. Journal of Ethnopharmacology, 2001, 78: 45-49.
7. Guevara BQ, Aguinaldo A, Espeso EI, Nonato M.G, Ysrael MC, Solevilla RC, et.al. A guidebook to plant screening: Phytochemical & Biological. Manila; UST publishing house:2004