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**Research Note:**

# BIOLOGICALLY ACTIVE CONSTITUENTS FROM *MENTHA Cordifolia* OPIZ LEAVES

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## ABSTRACT:

Isolates from yerba buena leaves were tested for their analgesic, antimutagenic, and anthelminthic activities. The acetic acid-induced writhing test showed that isolates β-sitosterol, β-sitosteryl-β-D-glucoside, and cis-8-pentadecenyllactone, at a dosage of 100 mg / kg mouse, each decreased the number of squirms induced by acetic acid by 70.0%, 73.0%, and 67.3%, respectively. β-sitosterol and an unsaturated carboxylic acid derivative are antimutagenic because they inhibited the mutagenicity of tetracycline by 65.3% and 68.7%, respectively, at a dosage of 0/01 mg / 20 g mouse, using the *in vivo* Micronucleus Test. *In vitro* tests using *Ascaris suum* showed that β-sitosterol is also anthelminthic as the behavior of worms approximate that of the positive controls, Combantrin and Antiox.

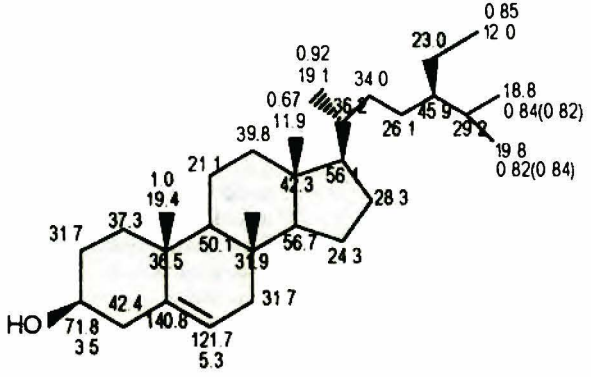
***Keywords:*** *Mentha cordifolia* Opiz., yerba buena, analgesic, anthelminthic, antimutagenic, β-sitosterol, β-sitosteryl-β-D-glucoside

*Mentha cordifolia* Opiz., commonly known as Philippine mint, marshmint, or yerba buena, is listed as one of the priority plants under the Department of Science and Technology (DOST)-Philippine Council for Health Research and Development (PCHRD)- National Integrated Research Program on Medicinal Plants (NIRPROMP). The unextracted and unpurified leaves are presently being produced in tablet form, including pediatric tablets, and have been proven as an analgesic in clinical trials phases I, II, and III (DOST Technical Report Series No. 12, 1991).

This paper is on the bioassay-directed isolation and structure elucidation of the bioactive constituents from yerba buena leaves. The leaves were extracted by immersing them in methanol. The methanolic extract was then solvent-partitioned into hexane, $CHCl_3$, and EtOAc extracts. Subsequent bioassay using the acetic acid-induced writhing test showed that the hexane extract is analgesic. *In-vitro* tests using live *Ascaris suum* as test animals showed that the hexane extract is also anthelminthic. An *in-vivo* Micronucleus Test showed that the $CHCl_3$ extract is

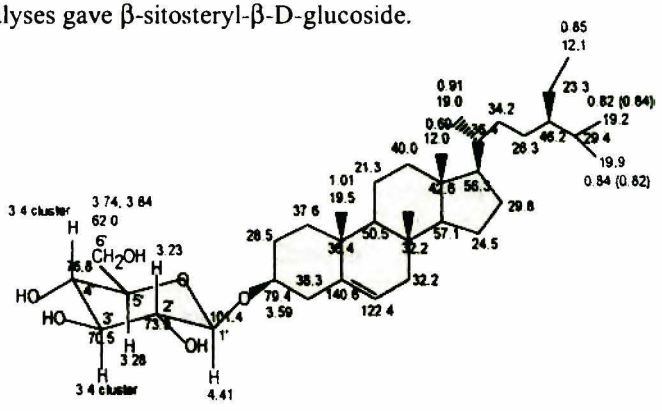
antimutagenic. The bioactive extract was then purified by sequential and repeated normal phase vacuum liquid chromatography using gradient elution. The structures of the bioactive isolates were elucidated by spectral analysis including gc-msd, ir, $^{1}H$- and $^{13}C$-nmr, COSY, HMQC, and HMBC.

The hexane fraction on sequential and repeated vacuum liquid chromatography (vlc)) over silica gel afforded the analgesic fractions labeled FB2 and FB10, eluted out at 20% EtOAc/ hexane and 30% EtOH/EtOAc, respectively. Vlc over silica gel of fraction FB2 yielded a greenish crystalline analgesic fraction which on recrystallization using ether/MeOH afforded white needle-like crystals 1 with an Rf 0.76, tlc (silica gel), EtOAc/hexane, 30:70, fuschia spot with vanillin-$H_2SO_4$. Spectral analyses gave β-sitosterol.




β-sitosterol

Fraction FB10 on vlc over silica gel 60G afforded 7 fractions with fraction FB10E, eluted from 8% MeOH/$CHCl_3$, exhibiting the highest analgesic activity. Vlc of FB10E yielded white crystals 2 upon recrystallization in ether/MeOH gave with an Rf 0.30, tlc (silica gel), MeOH/$CHCl_3$, 10:90, fuschia spot with vanillin-$H_2SO_4$. Spectral analyses gave β-sitosteryl-β-D-glucoside.




β-sitosteryl -β-D-glucoside

Confirmatory bioassay showed that β-sitosterol is analgesic, anthelminthic, and antimutagenic (Fig. 1, 2, 3). Its glucoside is also analgesic.

The analgesic cis-8-pentadecenyllactone is a white crystalline solid which degrades at 135.1°C. The antimutagenic unsaturated carboxylic acid is a yellowish crystalline solid which degrades at 210.8°C.

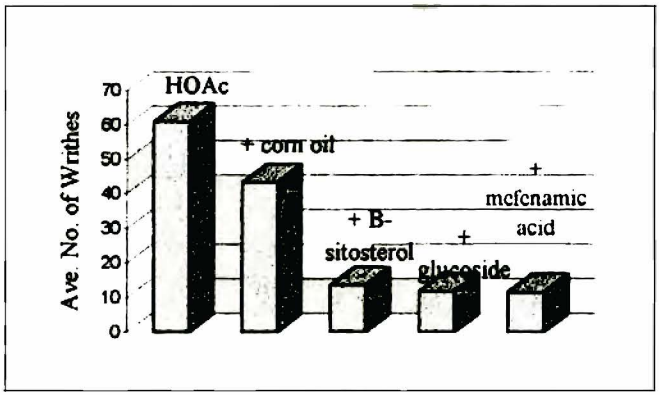



Figure 1. Analgesic activity using the acetic acid – induced writhing test

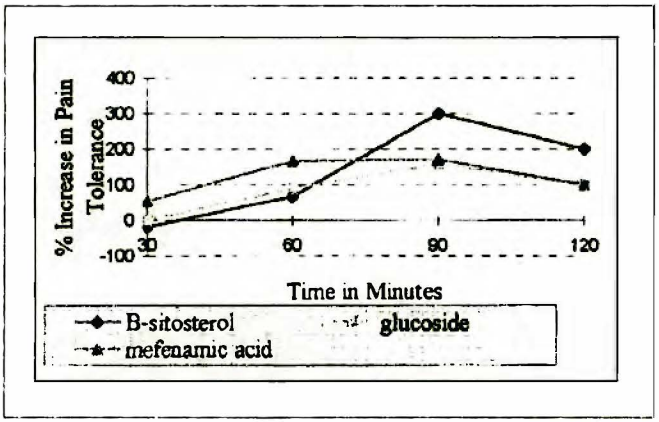



Figure 2. Analgesic activity using the hot plate method

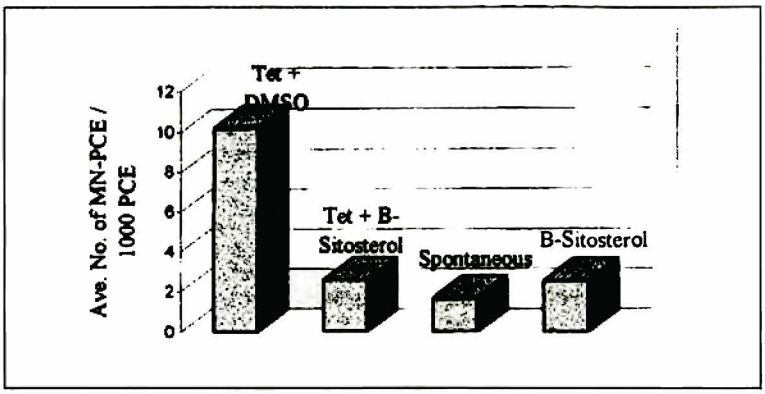



Figure 3. Antimutagenicity and mutagenicity using the micronucleus test