Journal of Medicinal Plants Research Vol. 3(10), pp. 822-824, October, 2009
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875© 2009 Academic Journals

Short Communication

Antisecretory and antiulcerogenic activities of the stem bark extract of *Melaleuca bracteata* and isolation of principles

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Accepted 10 August, 2009

In the present study, we have examined the effects of an ethyl acetate extract of *Melaleuca bracteata* on gastric acid secretion and indomethacin-induced ulceration. The plant is popularly known for its ornamental use in South Africa. Gastric acid secretion was studied using modified method of continuous acid secretion while gastric ulcers were produced in albino rats by oral administration of indomethacin. Results showed that, the extract significantly reduced gastric acid secretion in a dose-dependent fashion ($P < 0.05$). Similarly, in the animals pre-treated with extract and misoprostol, the mean ulcer scores of 4.90 ± 0.98 and 6.90 ± 0.60 respectively were significantly lower as compared to 13.42 ± 1.69 of the control ($P < 0.05$). This study also reports the isolation of 3-hydroxyl-lup-20 (30)-ene-28-oic (betulinic acid, BA), 3-hydroxylolean-12-ene-28-oic (oleanolic acid, OA) and its acetate from *M. bracteata* extract. The presence of BA and OA in the extract may account for its anti-ulcer effect.

Key words: *Melaleuca bracteata*, myrtaceae, gastroprotection, betulinic and Oleanolic acids.

INTRODUCTION

Melaleuca bracteata F, Muell belongs to the family Myrtaceae, which contains over 250 plant species. This plant, commonly called the tea tree or Johannesburg gold in South Africa, is popularly used as ornamental plant and well known for its vast medicinal properties. The plant's leaf is used to make poultices for infected wounds and skin disorders. It aids in stimulating glandular secretions, and reducing congestion in the vein. The leaves from the plant constitute a component of anti-HIV concoction used in Zululand by local medical practitioners in South Africa (Hutchings et al 1996). Many investigators have examined the essential oils of Melaleuca species. Penfold and Morrison (2002) reported that the oil from *M. bracteata* is a source of methyl eugenol and possesses insect repellant activity. Oils of the leaves of Melaleuca species (methyl eugenol from *M. bracteata*; 1, 8-cineole from M. quinquenervia and terpinen-4-ol from M. armillaris) are of economic importance (Aboutabl et al., 1990). According to Wilkinson and Cavanagh (2005), oils from M. alternifolia and Eucaliptus species are valuable antimicrobial agents for use alone or incorporated into cosmetics, cleaning agents and pharmaceutical products. Melaleuca species are known to have high stress tolerance, which is attributable to their ability to accumulate large quantities of osmoprotectants or proline (betaine) analogues (Naidu, 2003). Not much has been reported on non-volatile bioactive phytochemicals from this plant.

The plant *M. bracteata* is not known, even among the South Africans for its potential as possible gastro protective and anti-ulcer agent. Isolation of BA in the extract actually prompted the assay. The objective of this study was to determine the effects of ethylacetate extract of *M. bracteata* on gastric acid secretion and indomethacin – induced ulceration in rats and also examine the non-volatile constituents of the extract.

MATERIALS AND METHODS

Kofler Hot-stage melting point apparatus, Mettler H18 balance, JEOL AX-505 mass spectrometer, one-dimensional NMR

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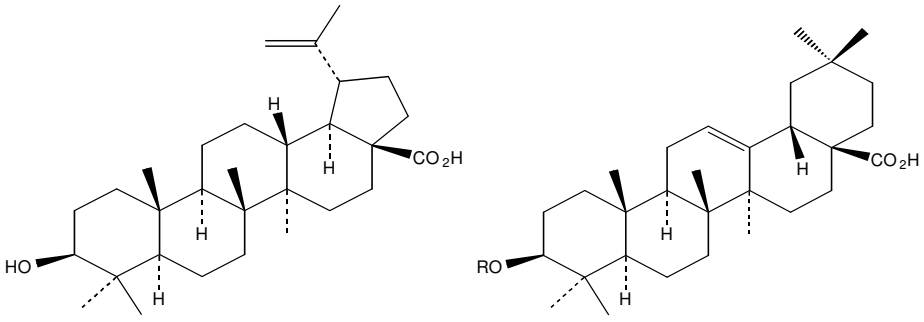

Figure 1. [1] Betulinic acid [2] Oleanolic acid (R = H) and [3] Oleanolic acid acetate (R = $COCH_3$).

(^{1}H:399.945 MHz; ^{13}C: 100.577 MHz), silica gel 60 (Merck 40 – 63 µm) for Column chromatography, pre-coated sheet silica gel 60-F^{254} (Merck) analytical TLC, visualized using UV (254 and 366) and vanillin-sulphuric acid spray.

Animals

Adult male albino rats weighing between 220 - 270 g of Wistar strain fed with normal rat cubes (Ladokun feeds, Nig. Ltd.) were used. They were kept under standard laboratory conditions. These animals were pre-treated with three doses (10, 20 and 40 mg/kg BW) of the extract for 7 days prior to the experiment. Standard ethical procedure for handling animals was observed.

Preparation of extract

The stem bark of *M. bracteata* F, Muell (Myrtaceae) was collected in front of a house in Durban town, South Africa where it served as ornamental tree. The plant material was authenticated by Professor H. Baijnath, School of Conservation & Biological Sciences-Westville campus, UKZN, Durban SA.

Dried pulverized stem back weighing 695g was extracted cold, successively with hexane and ethyl acetate. This afforded ethyl acetate (EtOAc) extracts (12.4 g). The percentage yield was 1.78%. The extract (2.3 g) was fractionated on an open column of Silica gel by gradient elusion with hexane (100%), hexane/EtOAc mixtures, EtOAc (100%) and EtOAc/Methanol mixtures. Fractions were collected in 50ml portions. Elution was monitored with analytical TLC in hexane/EtOAc (4:1), sprayed with H_2SO_4/vanillin reagent. Fractions with similar TLC pattern were combined. Purification of fractions15 - 16 yielded 3-hydroxylolean-12-ene-28-oic acid acetate [3], 20 - 44 gave 3-hydroxylolean-12-ene-28-oic acid (OA) [2], and 49-52 afforded 3-hydroxyl-lup-20 (30)-ene-28-oic acid (BA) [1]. The spectra data for BA, OA and its acetate were consistent with literature reports[2, 12]. They were thus identified by their spectroscopic data; ^{1}H-NMR; MS and ^{13}C-NMR and also comparison with authentic samples of OA and BA. The spectra are available with correspondence author (Figure 1).

Gastric acid secretory study

The modified method of Ghosh and Shild (1958) was used to measure gastric acid secretion. 24 h before the start of the experiments, feeds were withdrawn while water was given freely. Anaesthesia was induced with urethane (0.6ml/100g weight of a 25% w/v solution) given intraperitoneally. Each animal was surgically prepared for gastric acid secretion assay as earlier described by Adesanwo et al. (2003). Gastric acid secretory responses to different doses (10, 20 and 40 mg/kg BW) of extract were examined. The basal gastric acid secretion was determined before the administration of the extract.

Indomethacin – induced ulceration study

The method of indomethacin-induced gastric ulceration adopted was that reported by Adesanwo et al (2003). Feeding of the animals was terminated 24h before experiment but allowed free access to water. The animals were randomly divided into three groups, one group which served as control was given 0.1 ml distilled water, a second group received misoprostol (reference drug at 20 µg /kg BW) orally for 7 days while the third group was given the extract at 40mg /kg BW orally also for 7 days. Gastric ulcers were induced with oral administration of indomethacin (40mg / kg BW) after treatment period. Four hours after indomethacin administration, the animals were killed by cervical dislocation and their stomachs removed, opened and examined for gastric ulceration. Assessment of ulceration was by the scoring technique described by Elegbe (1978).

Statistical analysis

Results were expressed as Mean ± SEM. The test of significance between control and experimental groups was performed using student't' test.

RESULTS

From Table 1, intravenous administration of the stem bark extract of Melaleuca bracteata significantly decreesed gastric acid output in a dose-dependent fashion from the basal of 3.80 ± 0.65 - 2.90 ± 0.45 (10), 2.70 ± 0.41 (20) and 1.80 ± 0.32 (40) respectively.

Table 2 shows that, animals pre-treated with Misoprostol and the plant extract produced significant anti-ulcerogenic activity in these animals subjected to indomethacin administration.

DISCUSSION

The gastric acid secretion experiment showed significant reduction with increasing dose of the extract (Table 1). In

Table 1. The effect of *M. bracteata* extract on gastric acid secretion in rats.

Extract (mg/kgBW)	Gastric acid secretion (ml)						Mean acid secretion (ml)	Acid conc. (µeq./10ml) [a]
	i	ii	iii	iv	v	vi		
Basal (0)	0.25	0.38	0.67	0.23	0.32	0.42	0.38 ± 0.065	3.80 ± 0.65
10	0.15	0.35	0.25	0.18	0.43	0.35	0.29 ± 0.045 *	2.90 ± 0.45*
20	0.15	0.33	0.30	0.15	0.40	0.30	0.27 ± 0.041 *	2.70 ± 0.41*
40	0.10	0.23	0.15	0.10	0.30	0.20	0.18 ± 0.032 *	1.80 ± 0.32*

* Significantly different from the control at $P < 0.05$.
[a] Acid concentration = 10 x Titre value.

Table 2. The effect of *M. bracteata* extract on indomethacin induced ulceration in rats.

Group	Treatment	Mean ulcer score ± S.E.M.
I	Control (water)	13.42 ± 1.69
II	Misoprostol pretreated animals	6.90 ± 0.60*
III	Plant extract pretreated animals	4.90 ± 0.98*

* Significantly different from the control at $P < 0.05$.

another study (Table 2), the extract gave a significant reduction in gastric ulceration induced with indomethacin in which activity was almost similar to the effect of the standard drug, misoprostol ($P < 0.05$). This gastroprotective action of the extract might be due to the presence of betulinic acid and oleanolic acid. These two principles isolated from the extract have been reported in earlier studies for their gastroprotective effects (Luis et al., 2002, Adesanwo et al., 2003). Biopharmacological effects of betulinic and oleanolic acids include; anti-inflammatory (Honda et al, 2000; Han et al, 1997), anti-tumour (Shibata, 2001), anti-oxidant (Zhang et al., 2001; Somova et al., 2003), anti-diabetogenic (Yoshikawa and Matsuda, 2000), anti-HIV (Kashiwada et al., 2000). The antiulcer effect observed in this extract might therefore be due to inhibition of gastric acid.

Interestingly, the plant Maleleuca bracteata is not known even among the South Africans for its potential as an anti-ulcer agent. These results therefore seem to suggest that betulinic, and or oleaolic acid containing materials may have potential antisecretory effect.

Future studies may focus on the mode of action of the extract and its constituents reported in this paper.

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