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CHARACTERIZATION OF THE GLUCOSINOLATES AND ISOTHIOCYANATES IN MALUNGGAY (*Moringa oleifera* L.) EXTRACTS AND DETERMINATION OF THEIR MYROSINASE ACTIVITY AND ANTICANCER PROPERTIES

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Abstract: The research investigated the properties of glucosinolates in malunggay (*Moringa oleifera* L*)* samples including a chemical analysis of the major components in the edible parts, and the activity of their hydrolytic products.

HPLC studies showed that boiled seeds contain the highest levels of glucosinolates among the samples analyzed. Glucomoringin [4-(α-L-rhamnopyranosyloxy) benzylglucosinolate] was identified by LC-MS analysis of both intact and desulfated samples as the major glucosinolate in the malunggay extract, confirming previously published work on Moringaceae. Further evidence for the identity of the major compound was seen in the hydrolysis products which were obtained from an optimized procedure. 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate and its acetylated derivative, 4-(4'-*O*-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocyanate were found as the major breakdown products in the reaction catalyzed by exogenous myrosinase. Significant amounts of isothiocyanates were detected in seeds and leaves. These may be inherent in the samples or may have been formed during a sample preparation step. The values however markedly increased when samples were made to undergo hydrolysis in the presence of an active hydrolytic enzyme myrosinase, as evidenced by the ability of its extract to catalyze the hydrolysis of sinigrin. The enzyme has a K_M of 0.032 mM and V_{max} of 0.932 g^{-1} min^{-1} for sinigrin.

The ability of malunggay extracts to scavenge free radicals was measured using the DPPH assay. In this study, the scavenging potential was seen to increase with increased amounts of extract. The bioactivity of the malunggay extracts was assessed by determining its cytotoxic properties towards normal (HDFn), colorectal cancer (HT-29) and breast cancer (MCF-7) cell lines. The *M. oleifera* extracts exhibited toxicity towards the HT-29 cell line. All extracts proved to be non-cytotoxic to HDFn as well as MCF-7 cell lines at the concentrations tested. Compared to colchicine however, lower toxicities towards normal cells were exhibited by the *M. oleifera* extracts.

Key Words: *Moringa oleifera*, glucosinolates, glucomoringin, isothiocyanates, cytotoxicity

1. INTRODUCTION

Glucosinolates are sulfur-rich, secondary metabolites which are exclusively found in plants belonging to the order Brassicales. These compounds have been found to exhibit a number of biological activities, foremost among which is the reduction of a number of types of cancer in humans.

Malunggay (*Moringa oleifera* L.), a popular tree in the Philippines now slowly emerging as a "miracle vegetable", has been found to have significantly high levels of glucosinolates. The predominant compound in the seeds has been identified as glucomoringin, an uncommon glucosinolate on which few studies have been made as it is most closely associated only with the genus Moringaceae.

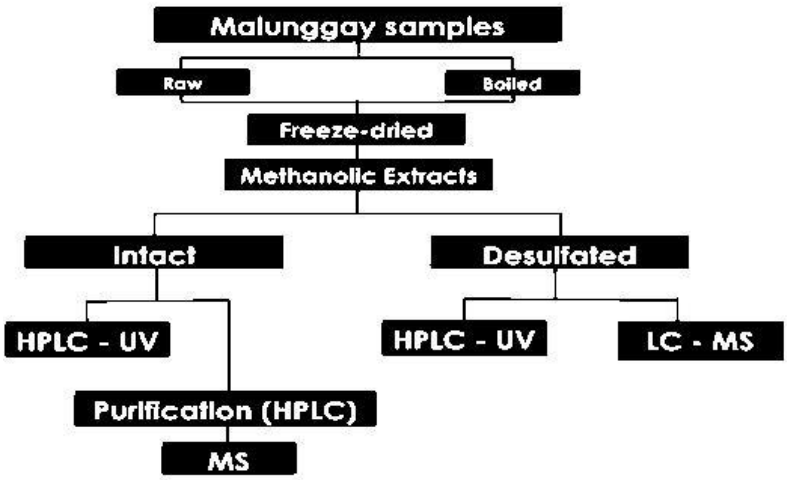
The present study investigated glucosinolates in edible parts (seeds and leaves) of malunggay. It involved qualitative and quantitative studies on the predominant glucosinolates and their hydrolytic products, the effect of traditional cooking (boiling) on these compounds as well as the potential chemopreventive properties which may be attributable to them.

Chromatographic methods (HPLC, LC-MS/MS, GC-MS) were employed in the quantitative analysis and identification of individual glucosinolates, isothiocyanates and other hydrolytic products. Assays involving cancer cell proliferation arrest, cancer cell death and DNA damage were also done to determine the anti-cancer properties of malunggay extracts.

2. METHODOLOGY

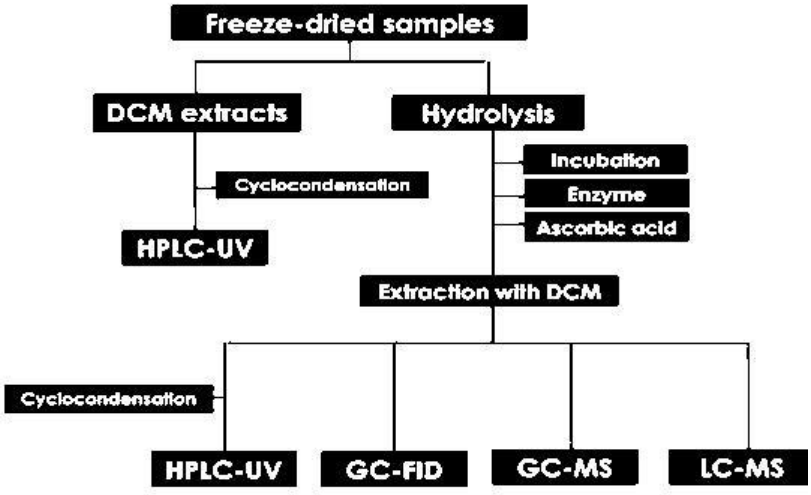
Analysis of Glucosinolates and Isothiocyanates

Glucosinolate concentrations of edible parts (seeds and leaves) of raw and boiled malunggay (*Moringa oleifera* L.) were determined by HPLC analysis of the desulfated derivatives. Characterization of the major glucosinolates was done by mass spectral analysis of the intact glucosinolate and LC-MS analysis of the desulfated derivative (Scheme 1).



Scheme 1. General Procedure for the Analysis of Glucosinolates

Certain aspects of glucosinolate hydrolysis in *Moringa oleifera* were investigated. These involved optimization of the hydrolysis procedure, identification of hydrolysis products and quantitative estimation of total isothiocyanate content by HPLC analysis of the cyclocondensation product.



Scheme 2. General Procedure for the Analysis of Isothiocyanates

Myrosinase Activity

The kinetic properties of malunggay myrosinase were determined by analyzing buffered (pH 6.5) assay mixtures containing varying amounts of sinigrin, the enzyme extract, and ascorbic acid. Samples were incubated for an optimum period prior to spectrophotometric analysis. The activity of the enzyme was calculated based on the decrease in the absorbance (at 227 nm) of the mixture due to the hydrolysis of sinigrin (Bellostas et al., 2008, Palmieri, et al., 1982).

Anticancer Properties of Malunggay Extracts

The effect of malunggay extracts on cell viability was evaluated using the PrestoBlue™ assay. Cells used in the determination of cell viability were HDFn (human dermal fibroblasts), HT-29 (human colon adenocarcinoma grade II) and MCF-7 (human breast adenocarcinoma). The cytotoxic and cytoprotective properties of malunggay extracts were compared against a positive control, colchicine.

3. RESULTS AND DISCUSSION

High performance liquid chromatographic (HPLC) analysis of desulfated derivatives of glucosinolates extracted from edible parts of *Moringa oleifera* revealed significantly high levels in both the seeds (696.94 µmol/g) and the leaves (368.89 µmol/g). Boiling increased glucosinolate levels in seeds but not in the leaves. This observation was attributed to the heat inactivation of hydrolytic enzyme in seeds and probable leaching out of leaf glucosinolates to the boiling medium. Differences in the spatial distribution of enzyme and substrate in leaf and seed cells may have influenced the observed changes in glucosinolate levels during cooking.

The major glucosinolate in malunggay was identified by LC-MS analysis of the desulfated derivative as well as the compound isolated in intact form by HPLC. Single ion monitoring of the peak at m/z 595 [M + Na^+] revealed fragmentations consistent with glucomoringin [4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate] *(1)* confirming previously reported findings on the plant (Fahey, et al., 2001).

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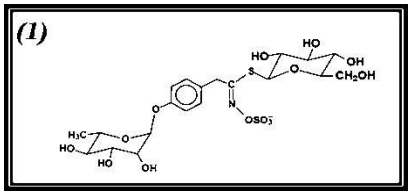
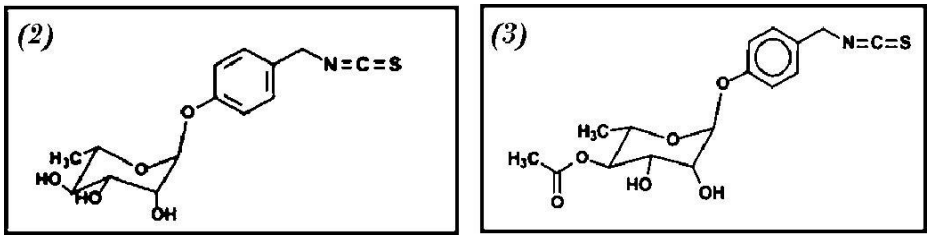


A hydrolytic process optimized for malunggay seeds yielded 4-(α-L-rhamnopyranosyloxy)

benzyl isothiocyanate *(2)* and 4-(4'-*O*-acetyl-α-L-rhamnopyranosyloxy) ***(3)*** , both of which are degradation products of glucomoringin and known to be present in malunggay (Kjer et al., 1979) The identities of these compounds were determined by LC-MS analysis and confirmed by comparison of mass spectral data with an isolate obtained from the same plant in a separate study (Ragasa, et al., 2012)) .



The total isothiocyanate contents of extracts of raw leaves and boiled seeds were determined by the reaction of the isothiocyanates with 1,2-benzenedithiol. The amount of product formed (1,3-benzenedithiole-2-thione) was quantified by HPLC. Significant amounts of isothiocyanates were found in unhydrolyzed samples but the concentration was found to increase considerably after hydrolysis in the presence of added enzyme.

M. oleifera contains an active hydrolytic enzyme myrosinase with K_M of 0.032 mM and Vmax of 0.932 g^{-} min^{-} for sinigrin.

The anticancer properties of malunggay were determined by chemical and biological assay. The free radical scavenging potential of malunggay measured using the DPPH assay was found to increase with increasing concentration of extract. PrestoBlue™ cell viability assays revealed that the extracts from malunggay (juice and hydrolysate) were non-cytotoxic towards normal cells (HDFn) but exhibited toxicity towards cancer cells (HT-29). IC_{50} values calculated for aqueous leaf extract, aqueous seed extract and seed hydrolysate were 10.74 mg, 7.53 mg and 1.308 mg, respectively. H_2O_2 - induced oxidative damage was prevented in normal cell lines by seed and leaf extracts.

The anticancer properties of malunggay may be attributed to the presence of bioactive isothiocyanates.

4. ACKNOWLEDGEMENT

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isothiocyanate also isolated from malunggay seeds.

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