Synergistic Anticancer Effects of Two Philippine Herbal Plant Extracts (*Momordica charantia* and *Psidium guajava*) on Spheroidal Cultures of a Filipino Patient-derived Lung Cancer Cell Line Elucidated

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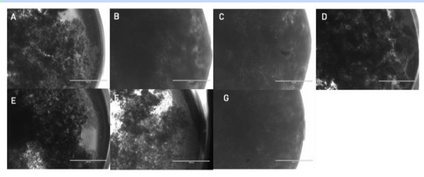
INTRODUCTION

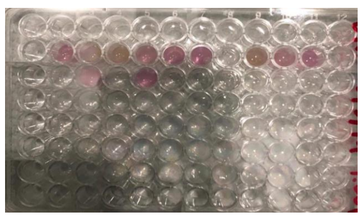
Globally, lung cancer is the leading cause of cancer-related deaths necessitating the development of alternative and effective treatment. Natural products have been a source of anticancer compounds with a potential to act in synergy to produce more robust therapeutic effects. Previous studies done on *Momordica charantia* and *Psidium guajava* demonstrated cytotoxic effects against A549 lung cancer cells[1]. The use of 2D cell cultures in these anti-cancer drug screening studies, however, fail to mimic the *in vivo* tumor microenvironment.

This study was able to determine the effects of the combinatorial treatment with *M. charantia* and *P. guajava* extracts on the expression of cancer-related genes in spheroidal cultures of a Filipino patient-derived lung cancer cell line (GL001 cells) and identify potential metabolites involved using *in silico* molecular docking simulations.



3D Spheroidal Culture of GL001 Cancer Cells

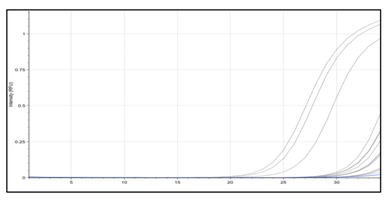
Spheroid cultures of Filipino patient-derived lung cancer cells were produced using the hanging drop method (250,000 cells/mL).



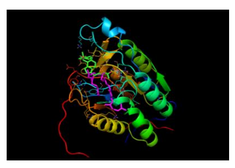
Treatment with Two Philippine Herbal Plant Combinatorial Extracts

M. charantia and *P. guajava* extracts were combined in a 3:2 ratio. Final concentration of the combined extracts for treatment is 1.46 ug/mL.

RT-qPCR of 3D Spheroidal GL001 Cancer Cells

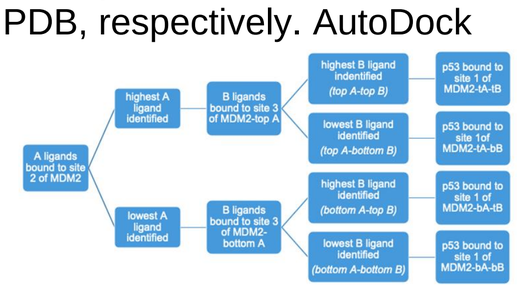


RNA was extracted from the GL001 cells and the mRNA expression of twelve (12) cancer-related genes was determined



Molecular Docking of Known Metabolites of Two Herbal Plants

Six metabolites for each plant were identified through *in silico* mining. The structures of the metabolites and receptors were obtained from PubChem and PDB, respectively. AutoDock Vina software was used to dock these structures.



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RESULTS

Gene Expression Analysis

RT-qPCR analysis of GL001 spheroidal cells upon treatment with combinatorial extract showed significantly different expression profiles for 6 out of 12 screened cancer-related genes for different pathways compared to non-treated controls (Table 1).

Table 1. Fold change expression of selected cancer-related genes upon combinatorial treatment.

Cancer Pathway	Gene	Fold Change
Apoptosis	*CASP8*	2.8879*
Cell Cycle	*MDM2*	0.2736**
DNA Damage	*ATM*	0.3439
Glycosylation	*NEU3*	0.3869
Epigenetics	*BRG1*	0.4175
Inflammation	*IL6*	0.6462

**highest fold change (upregulation)*
***lowest fold change (downregulation)*

Molecular Docking Analysis

CASP8 and *MDM2* were selected as receptors for molecular docking analysis since combinatorial extract respectively upregulated and downregulated their expression the most based on the RT-qPCR results. The binding affinity of Bid to CASP8 (Table 2) and the binding affinity of p53 to MDM2 (Table 3) were changed upon simultaneous docking of certain *M. charantia* and *P. guajava* metabolite combinations at two (2) different receptor sites. The metabolite combinations were selected based on binding affinity results from prior docking simulations.

Table 2. Binding affinities (kcal/mol) of Bid to CASP8 with or without the presence of *M. charantia* metabolites (α-eleostearic acid, Cucurbitacin B) and/or *P. guajava* metabolites (β-caryophyllene oxide).

Mode		Binding Affinity
Mode 1 (Bid only)		**-10.3**
Mode 3 (Bid, *P. gujava* at Site A, *M. charantia* at Site B)	β-caryophyllene oxide	-10.3
	β-caryophyllene oxide, Cucurbitacin B	-10.3*
	β-caryophyllene oxide, α-eleostearic acid	-10.3*

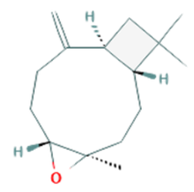
***synergistic metabolites w/ stabilizing effect*

The synergistic effect of certain combinations of *M. charantia* and *P. guajava* metabolites potentially involves a stabilizing effect on Bid-CASP8 interaction, which subsequently promotes apoptosis.

Table 3. Binding affinities (kcal/mol) of p53 to MDM2 with or without the presence of *M. charantia* metabolites (Kuguaglycoside C, α-eleostearic acid, Kuguacin J, Cucurbitacin B) and/or *P. guajava* metabolites (β-caryophyllene oxide, Kaempferol, γ-sitosterol).

Mode		Binding Affinity
Mode 1 (p53 only)		**-11.9**
Mode 2 (p53, *M. charantia* at Site A, *P. guajava* at site B)	Kuguaglycoside C	-9.9
	Kuguaglycoside C, β-caryophyllene oxide	-9.9*
	Kuguaglycoside C, Kaempferol	-9.9*
	α-eleostearic acid	-9.9
	α-eleostearic acid, γ-sitosterol	-9.7**
	α-eleostearic acid, Kaempferol	-9.9*
Mode 3 (p53, *P. guajava* at Site A, *M. charantia* at Site B)	γ-sitosterol	-10.2
	γ-sitosterol, Kuguacin J	-10.2*
	β-caryophyllene oxide	-11.9
	β-caryophyllene oxide, Cucurbitacin B	-9.9**
	β-caryophyllene oxide, α-eleostearic acid	-11.1**

**synergistic metabolites w/ stabilizing effect*
***synergistic metabolites resulting in decreased binding affinity*



On the other hand, the synergistic effect on p53-MDM2 interaction resulted in a decreased binding affinity between p53 and MDM2, which consequently promotes tumorigenesis. Additionally, specific metabolite combinations have potentially induced a synergistic stabilizing effect on ligand-MDM2 interactions, which then prevented the binding of other compounds that may promote p53-MDM2 binding.

CONCLUSION

This study has shown that *M. charantia* and *P. guajava* extracts have synergistic anti-cancer effects against a Filipino patient-derived cell line (GL001 cells) via upregulation of *CASP8* (*i.e.,* a pro-apoptotic gene) and downregulation of *MDM2* (*i.e.,* a proto-oncogene involved in cell cycle arrest). Furthermore, *in silico* molecular docking analysis revealed that specific metabolites from the two plants may mediate the said anti-cancer mechanisms. This work provides new opportunities through which plant-derived metabolites and molecular based therapeutics may be utilized for the treatment of lung cancer.

REFERENCES De Sagon, SP., Manansala, CM., Agapito, JD., & Heralde, FM., (2017). Cytotoxicity of Ten Philippine Medicinal Plants (10HG) on the A549 Lung Carcinoma Cell Line (Unpublished undergraduate thesis). University of the Philippines Manila, Manila, Philippines.; Ismail, N. I., Othman, I., Abas, F., H Lajis, N., & Naidu, R. (2019). Mechanism of apoptosis induced by curcumin in colorectal cancer. I*nternational journal of molecular sciences, 20*(10), 2454.