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Original Article

Antibacterial and Cytotoxic Activity of Selected Raw-Consumed Vegetables in West Java, Indonesia

Tiana Milanda[1], Raden Maya Febriyanti[2,*], Arif Satria Wira Kusuma[3], Ajeng Diantini[4]

ABSTRACT

Raw-consumed vegetables, known as *lalapan*, is famous among Sundanese community in West Java because of their heath benefits. In the present study, nine selected raw-consumed vegetables, namely *Vigna unguiculata, Ocimum tenuiflorum* Linn, *Psophocarpus tetragonolobus, Etlingera elatior, Abelmoschus esculentus, Oenanthe javanica, Centella asiatica, Pluchea indica* and *Pilea trinervia* were screened for their antibacterial and cytotoxic activity. Antibacterial activity test were conducting using disc diffusion method against *Serratia marcescens, Escherichia coli, Enterobacter cloacae.* Whereas, the cytotoxic activity were examined using WST assay against lung cancer cell line A549. For the antibacterial activity, this study finds that *Ocimum tenuiflorum, Etlingera elatior* and *Pluchea indica* have highest inhibition zone against tested bacteria. Furthemore, the results of the cytotoxicity assay indicated that among the nine plants tested, five plants showed IC_{50} < 20 µg/mL, including *Vigna unguiculata, Ocimum tenuiflorum* Linn, *Etlingera elatior, Centella asiatica* and *Pilea trinervia* with the IC_{50} value 13.71 µg/mL, 7.43 µg/mL, 12.45 µg/mL, 5.51 µg/mL and 18.84 µg/mL respectively.

Key words: Cytotoxicity, Antibacterial, Edible plants, West Java.

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INTRODUCTION

In recent years, the trend towards of the exploration of functional ingredients from natural dietary such as fruits, vegetables and spices for health benefit is experiencing rapid development. Study reveals that nutrition and healthy diet patterns can potentially strengthen the immune response against infections, cancers and non-communicable diseases.[1,2] Healthy diets such as Mediterranean diet, Veg and Japanese have been proven to reduce the risk of developing several cancers.[1]

People in West Java are known for their eating culture consuming raw (fresh) vegetables with their meals.[3,4] These facts are in favor with the renewed interest in research on plant-based medicines. Raw-consumed vegetables, known as *lalapan* in West Java, have been proven to provide many health benefits and potential in cancer prevention.[5] Study conducted by Amrinanto *et al.* (2019)[3] reveals that Sundanese dietary habits improve vegetables consumption and have a positive association with skin quality, β-carotene intake and blood β-carotene level.

Among many plants, basil (*Ocimum tenuiflorum* Linn), winged bean (*Psophocarpus tetragonolobus*), centella (*Centella asiatica*), cowpea (*Vigna unguiculata*), *beluntas* leaves (*Pluchea indica*), tespong herbs (*Oenanthe javanica*), *katuk* leaves (*Sauropus androgyhus*), *honje* (*Etlingera elatior*), pohpohan leaves (*Pilea trinervia*), cabbage (*Brassica oleracea* L), green eggplant (*Solanum melongena L*) and watercress (*Nasturtium officinale* R.Br.) are the mostly consumed plants as *lalapan* in West Java.[4]

The present study was conducted to screen the antibacterial and cytotoxic activity of extracts of vegetables which are consumed as *lalapan* by Sundanese community in West Java. In this study, antibacterial property was examined using the disc diffusion method against *Serratia marcescens, Escherichia coli, Enterobacter cloacae.* Cytotoxic activity of the extracts against lung cancer cell line A549 was examined using WST assay method which is based on the reduction of tetrazolium salt by NADPH enzyme.

MATERIALS AND METHODS

Materials

Fresh raw-consumed vegetables including basil (*Ocimum tenuiflorum* Linn), winged bean (*Psophocarpus tetragonolobus*), centella (*Centella asiatica*), cowpea (*Vigna unguiculata*), *beluntas* leaves (*Pluchea indica*), tespong herbs (*Oenanthe javanica*),), *honje* (*Etlingera elatior*), pohpohan leaves (*Pilea trinervia*) and okra (*Abelmoschus esculentus*) were collected at different localities around Jatinangor. The plants were identified at Herbarium of Biology Department, Faculty of Mathematics and Science, Universitas Padjadjaran. Lung cancer cell line A549 from American Type Culture Collection (ATCC) was the collection of Culture Cell Laboratory, Faculty of Pharmacy Universitas Padjadjaran. Cells were maintained in RPMI 1640 containing 100 units/mL penicillin and 100 mg/mL streptomycin and supplemented with heat-inactivated 10% fetal bovine serum (FBS). Tested bacteria *Serratia marcescens, Escherichia coli, Enterobacter cloacae* were the culture collection of Microbiology Laboratory, Faculty of Pharmacy Universitas Padjadjaran.

Preparation of extraction

Fresh vegetables were dried and then extracted using ethanol 96% with maceration method. The diluted extracts were then concentrated using a rotary evaporator and later on the waterbath till the crude

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plant extract is obtained. The extract was kept in the refrigerator for further use.

Phytochemical screening

The test of qualitatively identify the presence of alkaloids, phenol, flavonoids, saponin, tannin and terpenoid followed the previously described methods.[6]

Antibacterial activity

Disc diffusion method to assess antibacterial activity was carried out following the standard method by the National Committee for Clinical Laboratory.[5-8] Each extract was weighed as much as 200 mg, 400 mg, 600 mg and 800 mg and then dissolved in 5% dimethyl sulfoxide (DMSO) to a volume of 1 mL thus concentrations 0.2, 0.4, 0.6 and 0.8 were obtained. 50 µL of each extract concentration was dripped onto a sterile paper disc (with diameter 6 mm). Paper discs were allowed to dry on an open sterile petri dish inside Laminar Air Flow (LAF). Using a spread-plate technique, 20 µL of the test bacterial suspension was inoculated on 20 mL of MHA in sterile petri dishes. Petri dishes are divided into 4 zones, then each paper disk filled with ethanol extract of each test plant is inserted into each zone. The petri dish was incubated in an incubator at 37 ° C for 18-24 hours, then the diameter of the zone of inhibition (ZOI) was measured using a calliper. All experiments were carried out in triplicates.

Determination of Minimun Inhibitory Concentration (MIC)

The method described by Shirazi *et al*. (2013) was adapted with little modification to determine the Minimun Inhibitory Concentration (MIC) of extracts against tested bacteria. The concentration of the last extract caused turbidity from all wells used was the Minimum Inhibitory Concentration (MIC) of the extract. Only those extract with the highest ZOI value were further analyzed for the MIC.

In vitro assay for cytotoxic activity (WST assay)

Cell proliferation assay of lung cancer cell line A549 were determined using WST assay. Cells ($1x10^4$/well) were plated into each 96 well plates and incubated for 24 hours under conditioned 37^0C. After the cells are confluent for the study, various concentrations of each crude extract which was dissolved in 1% DMSO was plated into each well and was incubated for 24 hours. Cell viability were determined using cell counting kit CCK-8 at the absorbance of 450 nm and 620 nm as a reference. The cell proliferation inhibition (CPI) was calculated by the following formula:

CPI rate (%) = (1 - OD_{450} of the treated cells / OD_{450} of the untreated cells) x 100.

RESULTS

Extraction and phytochemical constituents' identification

The extract yield from the dried vegetables samples are varies between 3.2-14.49%, in which *Okra* seed pods (*Abelmoschus esculentus*) extraction yielded the most extract (Table 1).

Phytochemical screening shows distinct patterns of chemical compositions in constituents of the extracts. A qualitative phytochemical analysis was performed for the detection of alkaloids, phenols, flavonoids, saponins, tannins and terpenoids. The results of phytochemical evaluations are shown in Table 2.

Antibacterial activity

Preliminary screening of the crude ethanol extracts of nine raw-consumed vegetables was performed qualitatively using the disc diffusion assay. In the present study only those bacteria which found to be sensitive to the extracts were reported. The average of ZOI measurements for each extracts are shown in Table 3.

Table 1: Extract resulted from the maceration of dried vegetables.

No.	Plant samples	Weight of Dried Vegetables (gram)	Weight of Extract (gram)	Extract Yield (%)
1.	Cowpea (*Vigna unguiculata*)	163.76	6.88	4.2
2.	Holy Basil (*Ocimum tenuiflorum*)	130.66	9.51	7.28
3.	Winged Bean (*Psophocarpus tetragonolobus*)	218.33	29.64	13.58
4.	*Honje* (*Etlingera elatior*)	226.86	9.19	4.02
5.	*Okra* pods (*Abelmoschus esculentus*)	175.59	25.46	14.49
6.	*Tespong* herbs (*Oenanthe javanica*)	72.96	2.37	3.2
7.	Centella herbs (*Centella asiatica*)	401.14	29.26	7.29
8.	*Beluntas* leaves (*Pluchea indica*)	53.32	6.03	11.3
9.	*Pohpohan* leaves (*Pilea trinervia*)	144.33	11.14	7.71

Table 2: Results of phytochemicals screening of plants extract.

No.	Plant extract	Family	Alkalaoid	Phenol	Flavonoid	Saponin	Tanin	Terpenoid
1.	*Vigna unguiculata*	*Fabaceae*	+	+	+	+	-	+
2.	*Ocimum tenuiflorum*	*Lamiaceae*	+	+	+	+	-	-
3.	*Psophocarpus tetragonolobus*	*Fabaceae*	-	+	+	-	-	+
4.	*Etlingera elatior*	*Zingiberaceae*	+	+	+	-	-	+
5.	*Abelmoschus esculentus*	*Malvaceae*	-	+	+	+	-	+
6.	*Oenanthe javanica*	*Apiaceae*	+	+	+	+	-	-
7.	*Centella asiatica*	*Apiaceae*	-	+	+	+	-	+
8.	*Pluchea indica*	*Asteraceae*	-	-	+	+	-	-
9.	*Pilea trinervia*	*Urticaceae*	+	+	+	+	-	+

Remark:
+: present
-: absent

Table 3: **Zone of inhibition of nine extracts against bacteria.**

Plant extract	Tested bacteria	Zone of inhibition (mm) in various concentration			
		0.2	0.4	0.6	0.8
Vigna unguiculata		0.75	0.75	0.76	0.76
Ocimum tenuiflorum	*Serratia marcescens*	**0.79**	**0.82**	**0.83**	**0.86**
Psophocarpus tetragonolobus		0.78	0.81	0.91	1.03
Etlingera elatior		**1.37**	**1.89**	**2.17**	**2.20**
Abelmoschus esculentus	*Escherichia coli*	0.73	0.75	0.84	0.84
Oenanthe javanica		0.00	0.89	0.95	1.01
Centella asiatica		0.48	0.63	0.74	0.81
Pluchea indica	*Enterobacter cloacae*	**0.94**	**1.23**	**1.29**	**1.81**
Pilea trinervia		0.75	0.77	0.84	0.85

Table 4: **Antibacterial activity of extracts with highest ZOI against tested bacteria.**

Extract concentration (%)	Extract		
	Etlingera elatior	Ocimum tenuiflorum	Pluchea indica L.
0,08	+	+	+
0,16	+	+	+
0,31	+	+	+
0,63	+	+	+
1,25	+	+	+
2,5	-	+	+
5	-	+	+
10	-	-	-
20	-	-	-

Remark:
+: turbidity observed
-: no turbidity observed

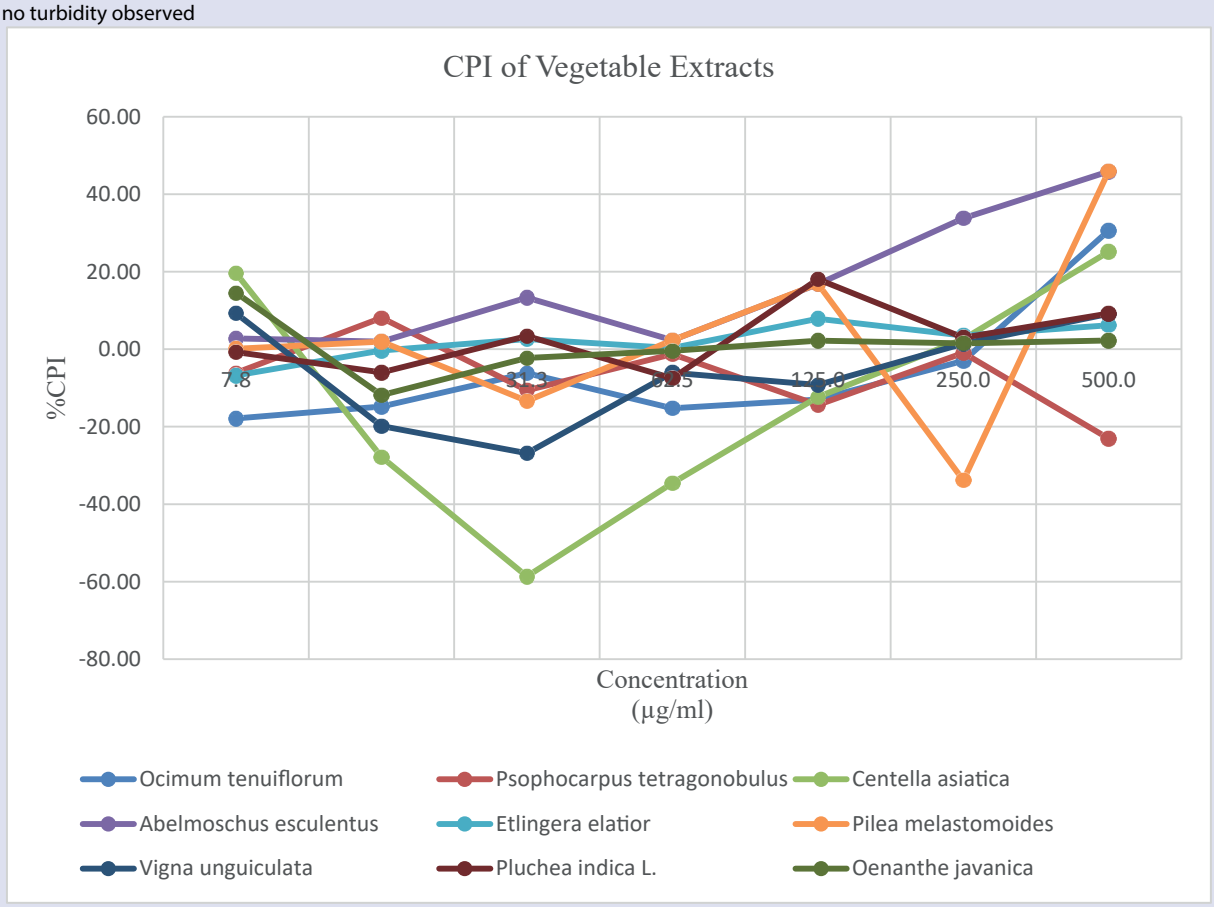

Figure 1: Dose response cell proliferation inhibition of nine extracts.

Table 5: IC_{50} value of samples in 24 hours observation.

No.	Plant samples	IC_{50} in 24 hours (µg/mL)
1.	***Vigna unguiculata***	**13,71**
2.	***Ocimum tenuiflorum***	**7,43**
3.	*Psophocarpus tetragonolobus*	92,12
4.	***Etlingera elatior***	**12,45**
5.	*Oenanthe javanica*	-88,92
6.	*Abelmoschus esculentus*	32,19
7.	***Centella asiatica***	**5,51**
8.	***Pilea trinervia***	**18,84**
9.	*Pluchea indica*	24,07

All of the samples displayed a concentration dependent of antibacterial activity. Furthermore, study found *Ocimum tenuiflorum, Etlingera elatior* and *Pluchea indica* have the highest ZOI value. Thus, those three extracts were further analysed for their MIC against tested bacteria.

Table 4 shows that MIC of *Etlingera elatior* against *Escherichia coli, Ocimum tenuiflorum* against *Serratia marcescens* and *Pluchea indica* against are 1.25 - 2.5% w/v, 5-10% w/v and 5-10% w/v respectively.

Cytotoxic activity

To date, there are still limited documentation about the identification of potential anticancer candidates from the raw-consumed vegetables by Sundanese community in West Java. In the present study, nine plant extracts derived from raw-consumed vegetables were screened for their cytotoxicity against human lung cancer cell line A549. To evaluate the cytotoxic activity of the extracts, lung cancer cell line A549 were treated with each plants extract in different concentration and observed for 24 hours. Dose response studies of all the extracts were summarized in the Figure 1.

IC_{50} calculation of nine plants extract in 24 hours observation revealed that 5 out of 9 plants extract give IC_{50} lower than 20 µg/mL. These plants included *Vigna unguiculata, Ocimum tenuiflorum* Linn, *Etlingera elatior, Centella asiatica* and *Pilea trinervia* (Table 5). Whereas *Psophocarpus tetragonolobus* and *Oenanthe javanica* extracts had no effect on A549 viability.

DISCUSSION

Several pathological condition such as cancer, infections and metabolic diseases have been associated with improper diet habit including less consumption of fruit and vegetables.[1,2] Furthermore, consumption of fruits and vegetables can be considered as an affordable strategy in diversification of healthy diet and reduce the development of non-communicable diseases.[2] Studies revealed that the empirical use of plants and vegetables for the prevention and treatment of skin disorders, inflammatory, infectious, parasitic and viral diseases has improve the chances for finding new, safe and effective anticancer agents as those conditions relevance to cancer or cancer-like symptoms.[9-11]

Sundanese community in West Java have rich cultural heritage and knowledge in the utilization of medicinal plants. They consume fresh edible plants as their dishes. Sundanese local dietary habits are characterised with high intake of fibres, less deep-fried and low saturated fats which have been associated with the positive effect on human health.[3,12] In the present study, nine of the most consumed vegetables as *lalapan* by people in West Java are subjected to in vitro test for their cytotoxic and antibacterial activities. Among nine plants, *Ocimum tenuiflorum, Etlingera elatior, Vigna unguiculata* and *Centella asiatica* show either one or both antibacterial and cytotoxic activity.

In the US NCI plant screening program, a crude extract is generally considered to have *in vitro* cytotoxic activity, if the IC_{50} value following incubation between 24 and 48 hours is less than 20 µg/mL (Kuete *et al*., 2013). It is noteworthy that *Vigna unguiculata, Ocimum tenuiflorum* Linn, *Etlingera elatior, Centella asiatica* and *Pilea trinervia* exhibited a significant (<20 µg/mL) activity against human lung cancer cell line A549. The cytotoxic activity of the extracts can be associated with the presence of secondary metabolites such as polyphenols, flavonoids and alkaloids.[13,14] Polyphenols and flavonoids have been collectively reported to possess anticancer properties such as antioxidants, inhibition of cancer cell growth, cytotoxic against cancer cell.[14] Moreover, flavonoids are reported to have various mechanisms as anti-lung cancer including activation of apoptosis, antiproliferative effects and cell cycle arrests.[15]

Our study found that *Centella asiatica* showed the highest cytotoxic against the lung cancer cell line A549. These results are supported with previous studies, although the analysis were detected on two different cell types. Previous study shown that *C.asiatica* showed a dose dependent inhibition against human breast cancer cell line MCF-7 and MDA MB 231.[16,17] Furthermore, recent study reports that triterpenoid isolated from the leaves of *C.asiatica* inhibited ionizing radiation-induced migration and invasion of A549 human lung cancer cells at noncytotoxic concentrations.[18]

Similar with *Centella asiatica, Ocimum tenuiflorum* was found to show high cytotoxicity against the lung cancer cell line A549. In addition, *O. tenuiflorum* also show high ZOI (0.79 mm) in a low concentration (0.2) against *Serratia marcescens*. *Ocimum tenuiflorum* or *Ocimum sanctum* from the family *Lamiaceae*, known as holy basil, is an edible plant native to Asia and Africa.[7,19,20] The antimicrobial properties of *O.sanctum* have been investigated in some previous.[8,21,22] The phytochemical screening of the extract of *O. tenuiflorum* showed the presence of flavonoids which is known for their anticancer properties.[23] Beside antibacterial and cytotoxic activity, *O. tenuiflorum* also exhibit several pharmacological activities including antioxidant, antidiabetic, antifungal, anti-lipid peroxidative and wound healing.[24]

Our study also found that *Etlingera elatior* exhibited antibacterial activity against *Escherichia coli* in low concentration with MIC 1.25 - 2.5% w/v. Moreover, *E. elatior* also cytotoxic against lung cancer cell line A549 with IC_{50} <20 µg/mL (12.45 µg/mL). *E. elatior* is native plant to Indonesia and Malaysia and widely cultivate throughout Southeast Asia. A study on its pharmacological activity, phytochemical and toxicity reported that the ethanolic extract is effective against *Pseudomonas aeruginosa, Bacillus megaterium and Escherichia coli* with the MIC are ranging from 1.56-50.0 mg/ml.[5] Other study reported that the various plants parts of *E. elatior* possess cytotoxic, antifungal and hepatoprotective activities. The extract displayed cytotoxic activity against HeLa with CD_{50} in the range of 10–30 µg/ml, MCF-7 and MDA-MB-231 with IC_{50} 173.1 and 196.2 µg/mL respectively.[25-27]

It is also noteworthy that Cowpea (*V.unguiculata*) has IC_{50} <20 µg/mL against human lung cancer cell line A549. *V.unguiculata* is one of the indigenous vegetables that is cultivated widely in Asia, Africa and South America.[28] Previous study reported that *V.unguiculata* shows high toxicity against HepG2, with LC50 56.4 mcg/ml.[29]

CONCLUSION

The screening results of the antibacterial and cytotoxic activity of nine raw-consumed vegetables in West Java showed that *Ocimum tenuiflorum, Etlingera elatior, Vigna unguiculata* and *Centella asiatica* show either one or both antibacterial and cytotoxic activity.

These findings provide the basis for the further investigation of each of these species and potential identification of novel bioactive compounds with antibacterial and anticancer properties. Furthermore, our study results also support the traditional knowledge and utilization on edible plants as sources of natural dietary supplements for maintenance of

health and wellness.

CONFLICTS OF INTEREST

The authors declare that there is no conflicts of interest, financial, or otherwise regarding the publication of this paper.

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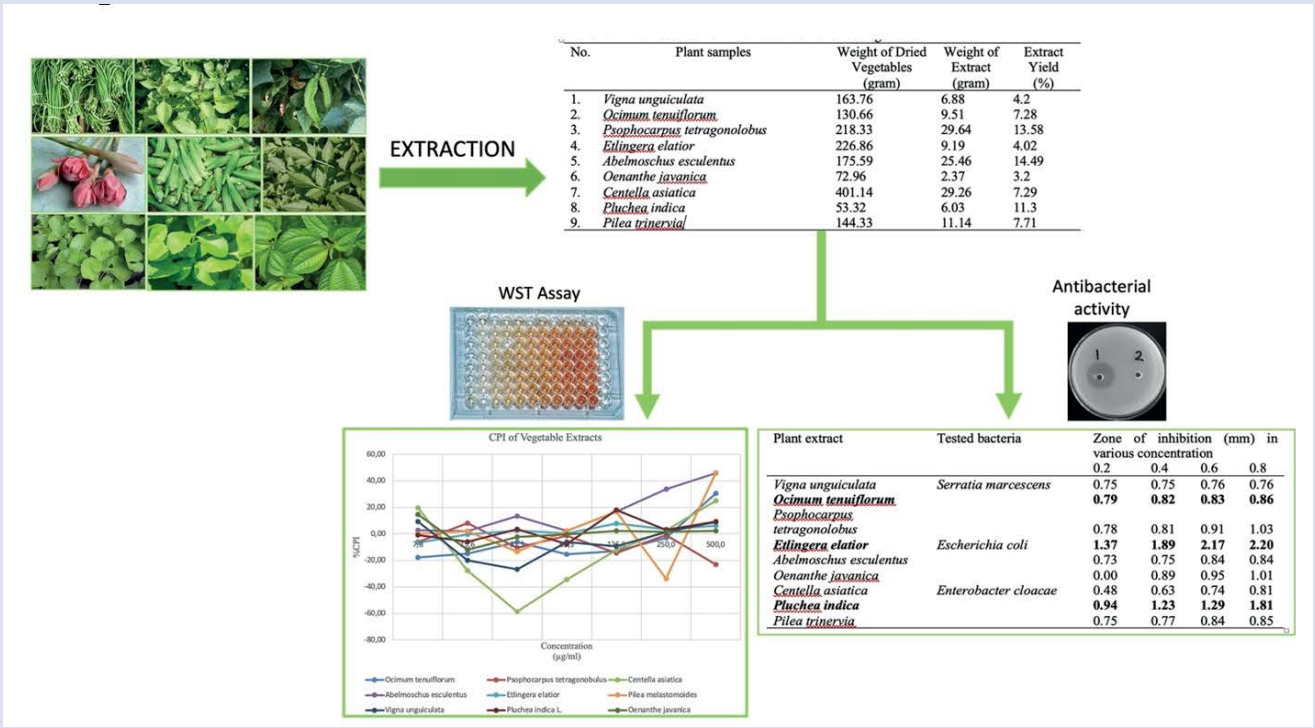
GRAPHICAL ABSTRACT



ABOUT AUTHORS

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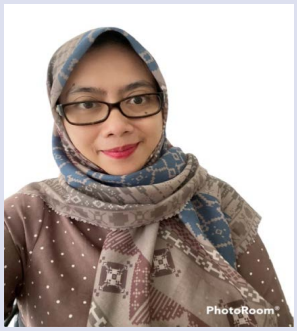

Doctor in the field of Ethnopharmacy and Lecturer at Department of Pharmaceutical Biology, Faculty of Pharmacy, Padjadjaran University, Jatinangor, Indonesia. Her study focused on the traditional use of medicinal, aromatic and cosmetic plants used by local community. Her current study is screening anticancer and antibacterial agents from medicinal plants using ethnodirected approach.

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Professor in Pharmacology and Clinical Pharmacy at Faculty of Pharmacy, Padjadjaran University, Jatinangor, Indonesia. Her study focuses in the non-pharmacological and pharmacological approach to improve the quality of life of cancer patients. Her recent study is the development of nutraceutical from edible plants.

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