RJC

RASĀYAN *J. Chem.*
Vol. 15 | No. 2 |1563-1566| April - June | 2022
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

ANTIOXIDANT AND CELL PROLIFERATION INDUCTION ACTIVITIES COMBINATION OF HYDROALCOHOL EXTRACT OF *Artocarpus lacucha* Buch. Ham. LEAVES AND *Anredera cordifolia* (Ten) Steenis. LEAVES

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ABSTRACT

The uncontrolled production of oxygen free radicals and the unbalanced mechanism of antioxidant protection results in the onset of many diseases, such as cancer, diabetes, Alzheimer's, heart diseases, and aging. *Artocarpus lacucha* Buch.-Ham. Belongs to the family of Moraceae, popularly regarded as a medicinal plant, and commonly called monkey jack. *Anredera cordifolia* (Ten.) Steenis also known as Binahong is a family of Basellaceae. Both the plants are widely used in traditional medicine. The aim of this study was to determine the antioxidant activity and proliferation activity of combination ethanol extract of *Artocarpus lacucha* leaves (EEAL) and *Anredera cordifolia* leaves (EEAC). The extract was prepared using ethanol 80% with the maceration method. Antioxidant activity was determined with the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. Proliferation activity was determined with an MTT assay. Antioxidant activity from combination with DPPH assay is measured as the percent of scavenging and the best combination is 37.5 µg/mL for EEAL and 12.5 µg/mL for EEAC. Proliferation activity in combination with MTT assay is measured as a percent of viability cells and the best combination is 37.5 µg/mL for EEAL and 37.5 µg/mL for EEAC. The results reveal that combination hydroalcoholic extract of *Artocarpus lacucha* Buch.-Ham. Leaves and *Anredera cordifolia* (Ten.) Steenis leaves have antioxidant and proliferative activity.

Keywords: Antioxidant, Cell Proliferation, Hydroalcohol, *Artocarpus lacucha* Buch. - Ham, *Anredera cordifolia (Ten.)* Steenis.

RASĀYAN *J. Chem.*, Vol. 15, No.2, 2022

INTRODUCTION

Oxidation is an important role in every organism. Free radicals producing from the metabolism process or environmental sources that interact simultaneously with the biological system. An electronic instability and highly reactive atoms or molecules are called reactive species. The diseases such as cancer, diabetes, Alzheimer's, heart diseases, and aging are caused by the uncontrolled production of oxygen free radicals and the unbalanced mechanism of antioxidant protection results.[1-3] Wound healing is a complex process involving many cells consisting of four phases namely hemostasis, inflammation, proliferation, and remodeling. The hemostasis, phase is the beginning of the wound healing process by involving platelets. During the inflammatory phase, fibroblasts function as cytokine secretions, and growth factors to activate the body's defense system. During the proliferation and remodeling phases, fibroblasts are important for granulating and reorganizing tissues of the extracellular matrix.[4-6] *Artocarpus lacucha* Buch-Ham belongs to the family of Moraceae, popularly regarded as a medicinal plant, commonly called monkey jack, and in Indonesia, it is called mobe. This plant is widely distributed in the tropical regions of south and south-east Asia, mainly Nepal, Srilanka, India, Myanmar, Indonesia, Vietnam, and Thailand. It has many pharmacological activities such as anti-inflammatory antiviral, anticancer, antibacterial, and anti-HIV. In

http://doi.org/10.31788/RJC.2022.1526442

Thailand, the dried aqueous extract of its heartwood has been used as a traditional anthelmintic agent.[7,8] *Anredera cordifolia* (Ten.) Steen is also known as Binahong is a family of Basellaceae, which is a medicinal plant that has been growing very well for a long. In Indonesia, the Binahong plant is still uncommon but in Vietnam, this plant has a high demand and is often used as a vegetable in Taiwan. In China and Taiwan, this plant is known to have tremendous benefits and has been consumed more than a thousand years ago. Almost all parts of the Binahong plant, such as tubers, stems, and leaves can be used in herbal therapy. Binahong leaf extract can stimulate fibroblasts and collagen formation, which accelerates the process of wound healing (9-10). The aim of this study was to determine the antioxidant activity and proliferation activity of combination ethanol extract of *Artocarpus lacucha* leaves (EEAL) and *Anredera cordifolia* leaves (EEAC).

EXPERIMENTAL

Preparation of Extract

The air-dried and powdered leaves of *Artocarpus lacucha* Buch. -Ham. And *Anredera cordifolia* Steenis. (500 g) was extracted by the maceration method with ethanol 80% (Merck). The filtrate was collected, and then evaporated under reduced pressure to give a viscous fraction and then dried to dry.[12-14]

Free Radical Scavenging Activity Test

The free radical scavenging activity was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH•) method. 0.2mM solution of DPPH• in methanol was prepared and 100µl of this solution was added to various concentrations of single and combination of *Artocarpus lacucha* Buch.-Ham.(EEAL) and *Anredera cordifolia* (Teen) Steenis. (EEAC). After 60 minutes, absorbance was measured at 516 nm.[2,12,15]

Analysis of Proliferative Activity

Single and combination of EEAL and EEAC (50 µg/mL; 37.5 µg/mL; 25 µg/mL; and 12.5 µg/mL) in *co-solvent* DMSO (Sigma) was submitted for proliferative test. In that way, the NIH-3T3 cell line ($1x10^4$cells/mL) was grown in DMEM complete medium. After 24h treatment, an MTT assay was performed and cell viability was counted to determine the proliferative activity.[16-18]

RESULTS AND DISCUSSION

Antiradical Activity

Antiradical power of the plant samples was measured in terms of hydrogen donating ability using DPPH which is a stable, nitrogen-centered free radical and produces deep purple color in methanol solution. Antioxidants either transfer an electron or a hydrogen atom to DPPH, thus neutralizing its free radical character [20]. DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. The DPPH assay has been largely used as a quick, reliable, and reproducible parameter to search the in vitro general antioxidant activity of pure compounds as well as plant extracts. The reducing capacity of compounds could serve as an indicator of potential antioxidant property[2,15,20] which is seen in Table-1.

Table-1: Scavenging percentage of extracts combination

No	% Scavenging	Concentration (µg/mL)	
		EEAL	EEAC
1.	25.84 ± 0.32	0	50
2.	61.78 ± 0.44	12.5	37.5
3.	71.88 ± 0.27	25	25
4.	78.50 ± 0.34	37.5	12.5
5.	68.73 ± 0.40	50	0

Proliferative Activity

The percentage of viable cells after treatment and incubation for 24h showed the stimulation effect of combination of the EEAL and EEAC towards the proliferation of NIH-3T3 cells. The percentage of viable cells after treatment with the best concentration combination of (EEAL and EEAC) 37.5 µg/mL and 37.5 µg/mL (125.44 ± 0.38%) showed the highest stimulation effect toward the proliferation of NIH 3T3 cells. The proliferative effect of the combination of EEAL and EEAC is given in Table-2.

Table-2: The proliferative effect of extracts combination

EEAL (µg/mL)	EEAC (µg/mL)			
	50	37.5	25	12.5
50	103.61 ± 0.47%	119.61 ± 0.37%	117.20 ± 0.58%	108.23 ± 0.82%
37.5	112.32 ± 0.87%	125.44 ± 0.38%	119.88 ± 0.48%	104.35 ± 0.37%
25	103.21 ± 0.44%	120.08 ± 0.64%	118.14 ± 1.05%	112.38 ± 0.48%
12.5	116.13 ± 0.70%	121.42 ± 0.55%	116.53 ± 0.42%	106.83 ± 0.55%

Proliferation is one of the crucial processes in the wound healing process especially during re-epithelization, as the fast proliferated fibroblast will provide a sufficient supply of cells to migrate rapidly and cover the wound site. Flavonoids are the compounds that can increase the proliferative process of fibroblast cells during the wound healing process such as apigenin, vitexin, isovitexin, orientin, and others [21-24].

CONCLUSION

The results reveal that combination hydroalcoholic extract of *Artocarpus lacucha* Buch.-Ham. Leaves and *Anredera cordifolia* (Ten.) Steenis leaves have antioxidant and proliferative activity.

ACKNOWLEDGMENT

We gratefully thank the Ministry of Research and Technology/ National Agency for research and Innovation through the "Hibah Penelitian Dasar Unggulan Perguruan Tinggi" research grant 2020 for financial support in the study.

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[RJC-6442/2021]