Phytochemical Analysis And Assessment Of The Free Radical Scavenging Activities Of The Extracts Of Artocarpus Ovatus Blanco (Moraceae) Leaves

Joseph Mari B. Querequincia, Marina O. Osi, Sandra C. Sy

Abstract: Artocarpus ovatus Blanco is an endemic plant species belonging to the family Moraceae. This study evaluated the free radical scavenging activities of the extracts from A. ovatus ethanolic leaves. The crude plant extract and its semi-crude extracts were screened for the presence of secondary metabolites using thin layer chromatography and UV-Vis spectrophotometry. In the phytochemical analysis, presence of secondary metabolites such as flavonoids, and polyphenols in the ethanol extract and DCM semi-crude extract were detected. In the in vitro antioxidant assays, the ethanol extract demonstrated a significant DPPH (IC50 = 0.078 mg/mL) and nitric oxide (IC50 = 0.045 mg/mL) radical scavenging activities as well as hydrogen peroxide (IC50 = 0.098 mg/mL) scavenging effect as compared with other sample extracts.

Index Terms: Antioxidant, Artocarpus ovatus, Moraceae, Flavonoids, Polyphenols, DPPH, Hydrogen peroxide, Nitric oxide

1 INTRODUCTION

Scientists globally conduct researches about alternative sources of treatments and these would come from medicinal plants. Plants that belongs to the Artocarpus genus comprise of 50 evergreen species belonging to the family Moraceae. Most of these plant species contains secondary metabolites compounds such as flavonoids, stilbenoids and triterpenes which have medicinal value. One of the popular plant species of the Artocarpus genus in the Asian region is the Artocarpus heterophyllus. There are a number of established pharmacological potentials of A. heterophyllus such as hypoglycemic, hypolipidemic, gastroprotective, antimicrobial and antineoplastic activities [1]. An endemic plant species of the said genus in the Philippines is Artocarpus ovatus Blanco locally known as Anubling. However fewer literatures on this plant species about its medicinal potentials are available. This study intends to characterize the presence of phytochemical compounds and establish the free radical scavenging activity of this plant species.

2 MATERIALS AND METHODS

2.1 Chemicals and reagents

Ethanol, 95% (RCI Labscan, Thailand), Dichloromethane (RCI Labscan, Thailand), Hexane (RCI Labscan, Thailand), n-Butanol (RCI Labscan, Thailand), Ascorbic acid (Sigma-Aldrich, Singapore), Folin-Ciocalteu (Sigma-Aldrich, Singapore) and 2,2-Diphenyl-1-picrylhydrazyl (Sigma-Aldrich, Singapore) were acquired from Bellman Corporation.

2.2 Plant collection, authentication and extraction

The fresh and matured leaves of Artocapus ovatus Blanco were collected from Buhi, Camarines Sur, Philippines and its sample species was authenticated by the curator of the Botany Division of the National Museum of the Philippines with a voucher specimen number 198528. A. ovatus leaves were air-dried for 14 days and was pulverized using a Thomas Wiley miller. The powdered leaves were weighed with the use of a Sartorius top loading balance instrument and then soaked in 95% ethanol in a percolator for 24 hours and the percolate was collected. The percolate was concentrated using an R-200 rotary evaporator (Buchi, Switzerland) under reduced pressure until a viscous consistency was obtained. The concentrated extract was further dried at 35 ° C kept in an amber colored glass at 0 to 8° C and was used for further analysis [2]. An aliquot of the crude extract was partitioned using the solvents of increasing polarity starting from hexane, dichloromethane and n-butanol. The collected semi-crude liquid extracts were concentrated under vacuo at 45° C [3].

2.3 Phytochemical testing

Thin layer chromatography was used to characterize the presence of flavonoids and polyphenols in the extracts of Artocarpus ovatus leaves. An amount of 5 mg extract samples were mixed with dimethyl sulfoxide. These dilutes samples were incorporated in the Merck TLC Silica gel 60 F_{254} pre-coated aluminum plates with dimensions of 20 x 50 cm. The solvent system, petroleum ether: ethyl acetate (6:2) to was utilized to develop the chromatogram. Visualizing spray reagents that was used were the potassium ferricyanide-ferric chloride and antimony chloride spray reagents. The chromatograms were visualized as well under 240 nm and 365 nm [4]. Total flavonoid and phenol contents were also determined using aluminum chloride and Folin-ciocalteu methods, correspondingly.

2.4 In vitro free radical scavenging assays

2,2-Diphenylpicrylhydrazyl (DPPH), hydrogen peroxide and nitric oxide radical scavenging assays were utilized to evaluate the in vitro antioxidant capacity assays of extracts from Artocarpus ovatus leaves. Absorbance readings of the reactions in 96-well microplate were done at 517 nm, 230 nm and 540 nm, respectively using an SH-1000 Corona Microplate Reader (Hitachi, Japan) [5]. Ascorbic acid was used as the standard.

- *Joseph Mari B. Querequincia (main author) is currently connected with the Pharmacy Department of San Pedro College, Davao City, Philippines, 8000.*
- *Marina O. Osi and Sandra C. Sy (co-authors) are affiliated with the graduate school and faculty of Pharmacy of the University of Santo Tomas, Manila, Philippines, 1015.*

2.5 Data interpretation and analysis

Results in the assays were expressed as IC50 values using four logistic parameter regression analysis using a licensed computer package Prism 7.0. based from the percent inhibitions computed. Raw data were subjected to initial analysis of variance and post hoc Duncan's test with a level of significance of 0.05.

3 Results and Discussion

3.1 Plant extraction yield

The ethanol extract has a yield of 17.901%. Among the semi-crude extracts, the DCM extract had the highest yield which is 10.710%. All of the extracts are dark green in color with viscous consistency.

3.2 Phytochemical analysis

Thin layer chromatography using petroleum ether: ethyl acetate (6:2) as the developing solvent system revealed the presence of flavonoids and polyphenols in ethanol extract (Potassium ferricyanide-ferric chloride spray reagent: Rf values = 0.17, 0.71, 0.79 and 0.86; antimony chloride spray reagent: Rf values = 0.17, 0.24, and 0.50) and DCM semi crude extract (Potassium ferricyanide-ferric chloride spray reagent: Rf values = 0.53 and 0.78; antimony chloride spray reagent: Rf values = 0.27 and 0.53). For the total flavonoid content, the ethanol extract yielded a considerable flavonoid content amounting to 13.57 ± 0.046 mg QE/g sample, followed by DCM semi-crude extract with a content of 11.52 ± 0.044 mg QE/g sample and the next is n-Butanol semi-crude extract with 4.24 ± 0.012 mg QE/g. In the total phenol content assay, the ethanol extract contains significant total phenol content with an amount of 22.92 ± 0.33 mg GAE/g sample, followed by DCM semi-crude extract of 17.54 ± 0.20 mg GAE/g sample and n-Butanol semi-crude extract with 15.11 ± 0.33 mg GAE/g sample. Hexane semi-crude extract have nil results.

3.3 In vitro free radical scavenging assays

Among the extract sample the one that has the least IC50 values in the different scavenging assay models was the ethanol extract which was followed by the DCM semi-crude extract. N-Butanol and hexane semi-crude extracts have high IC50 values in which they have the least scavenging activity. The IC50 values represent the amount of the extract samples needed to inhibit 50% of the free radicals. The minimal the value the more potent the sample on its action on inhibiting free radicals [6]. Please refer to tables 1, 2 and 3 for the IC50 values of the extract samples on the different scavenging assays. The n-butanol and hexane fractions have insignificant results. Their scavenging effects of both the ethanol extract and the DCM semi-crude extract might be attributed to the presence of polyphenols and flavonoids in these extract samples. The said phytochemical compounds act as reducing agents and electron donors that are capable of eliminating free radicals. These free radicals can cause disturbances in normal physiological processes [7]

TABLE 1

IC_{50} VALUES OF THE SAMPLES AGAINST DPPH RADICALS

Sample	IC_{50} values (in mg/mL)
Ascorbic acid	0.016 mg/mL
Ethanol extract	0.078 mg/mL
Hexane semi-crude extract	> 0.5 mg/mL
DCM semi-crude extract	0.201 mg/mL
n-Butanol semi-crude extract	0.379 mg/mL

TABLE 2

IC_{50} VALUES OF THE SAMPLES AGAINST NITRIC OXIDE RADICALS

Sample	IC_{50} values (in mg/mL)
Ascorbic acid	0.001 mg/mL
Ethanol extract	0.045 mg/mL
Hexane semi-crude extract	> 0.5 mg/mL
DCM semi-crude extract	0.130 mg/mL
n-Butanol semi-crude extract	> 0.5 mg/mL

TABLE 3

IC_{50} VALUES OF THE SAMPLES AGAINST HYDROGEN PEROXIDE

Sample	IC_{50} values (in mg/mL)
Ascorbic acid	0.020 mg/mL
Ethanol extract	0.098 mg/mL
Hexane semi-crude extract	> 0.5 mg/mL
DCM semi-crude extract	> 0.5 mg/mL
n-Butanol semi-crude extract	> 0.5 mg/mL

4 Conclusion

Artocarpus ovatus ethanol extract and its DCM semi-crude extract contains secondary metabolites such as flavonoids and polyphenols. They exhibited considerable in vitro free radical scavenging capacities against DPPH, nitric oxide radicals and hydrogen peroxide molecules having least IC50 values.

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