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Bioprospecting of non-timber forest products of selected flora in Baluran National Park, East Java, Indonesia for phytochemicals, anti-diabetic, anti-tyrosinase, and antioxidants

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Abstract. *Ramadhan R, Ni'matuzahroh, Lestari DA, Hariyanto NA, Suwandari S, Kurnia IT, Phuwapraisirisan PP, Hasan MI, Phontree K, Setiawan J, Salamun, Wahono ND, Supriyanto A, Affandi M. 2024. Bioprospecting of non-timber forest products of selected flora in Baluran National Park, East Java, Indonesia for phytochemicals, anti-diabetic, anti-tyrosinase, and antioxidants. Biodiversitas 25: 3803-3815.* The ecosystem within Baluran National Park comprises elements that exhibit varying degrees of sensitivity to environmental influences, encompassing both biotic and abiotic factors. It has been observed that identical plant species may display significant variations in their secondary metabolite content across different habitats. The primary objective of this investigation is to analyze the phytochemical composition, antioxidant properties, anti-tyrosinase effects, and anti-diabetic potential of specific flora species found in Baluran National Park. Furthermore, the research aims to identify novel natural resources with anti-diabetic and antioxidant properties among the selected flora from Baluran National Park, East Java, using chemotaxonomic and ethnopharmacological data. The study involved the qualitative and quantitative analysis of phytochemicals, along with assessing their in vitro anti-diabetic, anti-tyrosinase, and antioxidant activities through various assays such as α-glucosidase inhibition, tyrosinase inhibition, and free radical scavenging tests (DPPH, ABTS, CUPRAC, and FRAP). The findings reveal that ethanol extracts of *Euphorbia atoto* demonstrated potent *α*-glucosidase inhibitory activity with a percent inhibition (PI) value of 92.07±1.43%, surpassing the inhibitory effects of acarbose (25.92±1.61%) and quercetin (84.79±1.18%). Additionally, the antioxidant assessments indicated that the selected flora from Baluran National Park exhibited substantial antioxidant properties, as evidenced by their percent inhibition (PI) values. These results provide a scientific basis for considering the flora of Baluran National Park as a potential source of natural medicinal compounds.

Keywords: Anti-diabetes, antioxidant, anti-tyrosinase, Baluran National Park, phytochemicals

INTRODUCTION

Indonesia is recognized as the second most biodiverse country globally, after Brazil, due to its significant wealth and variety of flora and fauna (Pasaribu et al. 2021). Baluran National Park in East Java exemplifies this biodiversity with its diverse ecosystems, including savanna, mangrove, coastal, mountain, swamp, and deciduous forests spread across 25,000 hectares (Sulasmi et al. 2019). The park's climatic conditions and geographical features support the growth of a wide range of flora akin to tropical forests in Africa. Baluran National Park is also known for its abundant biodiversity, including various medicinal plants (Zahra et al. 2020). Throughout history, medicinal plants and traditional medicine have played an integral role in promoting health, enhancing endurance, and managing illnesses. As a result, these practices continue to hold significant importance in the lives of certain individuals to this day (Gumisiriza et al. 2019). Indonesia's lush tropical forests harbor a plethora of medicinal plant species and are home to diverse ethnic communities with unique traditional medical expertise. The country boasts numerous uncultivated medicinal plants, relying on natural sources for their availability. Forest resources can be categorized into two main groups: wood

forest products and their by-products, known as timber products, and non-timber forest products (NTFPs) (Shackleton et al. 2018). Among the non-timber forest products (NTFPs) commonly utilized by local communities near forests are medicinal plants (Pasaribu et al. 2021; Haqqin et al. 2024). Numerous biodiversity elements associated with non-timber forest products (NTFPs) within Baluran National Park, including mushrooms, ferns, fruits, and medicinal plants, have not been thoroughly examined for their pharmacological potential. The medicinal plant species in Baluran National Park are important for planning further research on phytochemical contents and their biological activities.

Non-timber forest products (NTFPs) encompass a range of items derived from forests and their primary sources, such as medicinal plants, honey, mushrooms, resins, fruits, nuts, vegetables, barks, and natural fibers. These products are utilized by local communities living in forests to sustain their livelihoods (Silva et al. 2020). Medicinal plants, a subset of NTFPs, have been historically employed in traditional medicine for treating various ailments. Given the historical reliance of humans on plants for sustenance, the utilization of plants for medicinal purposes dates back to early civilizations (Moukette et al. 2015). Medicinal plants are recognized for their diverse bioactive secondary metabolites, including phenolics, flavonoids, alkaloids, terpenes, and tannins. These compounds, with their therapeutic properties such as anti-diabetic, antioxidant, anti-inflammatory, wound-healing, and antibacterial effects, are a fascinating area of study and a potential source of future medical breakthroughs (Rozirwan et al. 2022).

Hyperglycemia, a common complication of diabetes mellitus, often arises from an imbalance in the body's insulin requirements. It is associated with various adverse effects, including diabetic foot, heart disease, renal failure, retinopathy, stroke, and complications during pregnancy (Xu et al. 2020). Several strategies have been developed to manage blood sugar levels in diabetes treatment, such as insulin secretagogues, insulin sensitizers, glucose recapture inhibitors, and antihyperglycemic agents. Research indicates that controlling postprandial blood glucose levels using antihyperglycemic medications that inhibit α-glucosidase can effectively manage the progression of diabetes (Trinh et al. 2016). Patients with type 2 diabetes can utilize synthetic drugs, such as acarbose, miglitol, and 1-deoxynojirimycin, which act as digestive enzyme inhibitors to reduce postprandial hyperglycemia and improve glucose tolerance. However, the use of these drugs is often associated with gastrointestinal issues (Chaudhury et al. 2017). Phytochemicals, a class of beneficial secondary metabolites present in medicinal plants, have demonstrated potential in treating hyperglycemia and its related complications by scavenging free radicals (Akyuz et al. 2022).

The selection of Baluran National Park as the research site was motivated by several factors, namely its proximity to protected areas, diverse biodiversity, varied ethnocultural composition, and the tradition of sharing indigenous knowledge among local inhabitants. In addition, the need for published data regarding the traditional medicinal plant knowledge utilized by the local communities to treat different illnesses was a significant factor in choosing this location. This indigenous knowledge, predominantly transmitted orally, is at risk of being lost over time due to insufficient documentation. Therefore, this study aims to investigate selected flora from Baluran National Park, which demonstrates dual functions, namely α-glucosidase inhibition and free radical scavenging activities. To the best of the authors' knowledge, this study represents the first evaluation of phytochemicals, anti-diabetic, anti-tyrosinase, and antioxidant activities of selected flora from the dry tropical forests of Baluran National Park.

MATERIALS AND METHODS

Plant collection and identification

Seventeen selected plant species were identified, and their fresh leaves were collected from Baluran National Park, located in Indonesia. The selection of these species was based on chemotaxonomic research and traditional knowledge from local communities regarding plants recognized for their medicinal properties and pharmacological significance (Figure 2). The collection of leaves occurred in March 2024 at Baluran National Park in Indonesia (Figure 1). The identification of the selected plant from Baluran National Park was carried out by the botanist staff of Baluran National Park by Mr. Nanang Tri Wahono. A voucher specimen was archived at the Exploration and Synthesis of Bioactive Compounds (ESBC) Research Group, part of the University Center of Excellence-Research Center for Bio-Molecule Engineering (PUI-PT BIOME) at Universitas Airlangga. The scientific names and dry extract yields of these plants are presented in Table 1.

Chemical reagents and instrument

ABTS (2,2`-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) and DPPH (1,1-diphenyl-2-picrylhydrazyl) were procured from Tokyo Chemical Industry Co. Ltd. Potassium persulfate, hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ammonium acetate, neocuproine, copper (II) chloride, α-glucosidase, tyrosinase from mushroom, p-NPG (4-nitrophenyl-α-D-glucopyranoside), L-DOPA (L-3,4-dihydroxyphenylalanine), acarbose, kojic acid and ascorbic acid were procured from Sigma Chemical Co (St. Louis, MO, USA), while all remaining chemicals and solvents utilized in the study were of premium commercial quality. Absorbance measurements were taken using a 96-well Thermo Scientific Multiskan SkyHigh RE 6.1.1 microplate reader.

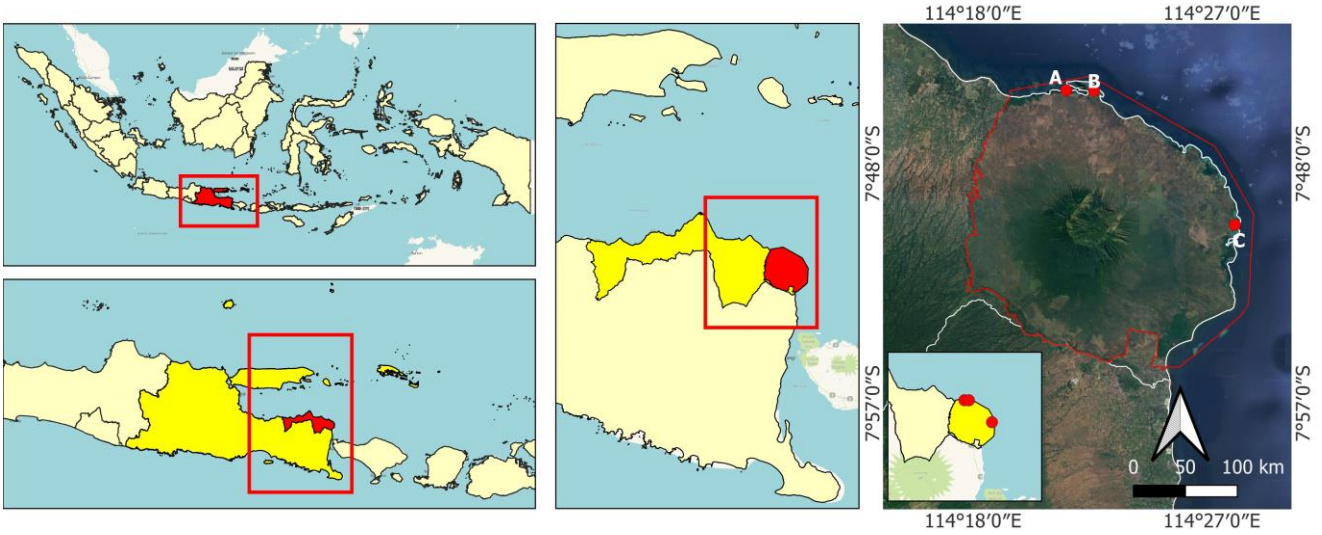

Figure 1. Selected plants of the study area in Baluran National Park, East Java, Indonesia (A. Pantai Sijile; B. Pantai Bilik; C. Pantai Bama), Situbondo, East Java, Indonesia

Extraction of medicinal plants

The plant leaves were rinsed with distilled water and air-dried in a well-ventilated room at room temperature for 4 days. The extraction of selected plants from Baluran National Park was performed according to the methods of Ramadhan et al. (2019). The dried material was finely ground using a grinder and then subjected to extraction processes using ethanol 96% (*v*/*v*) (3 times × 400 mL each). The resulting mixtures were macerated at room temperature and filtered through filter papers. The filtrate was evaporated under vacuum conditions using a rotary evaporator (Rotavapor R100, BUCHI) to yield a dry crude extract. This extract was transferred into amber containers and stored at 4°C for future bioassay analysis.

Phytochemicals analysis

A phytochemical analysis was conducted following the methodology established by Owusu-Ansah et al. (2023) and Seck et al. (2021) to identify various chemical constituents, including alkaloids, flavonoids, saponins, phenolics, and terpenoids. Details on the detection of each chemical group are provided in the following sections.

Alkaloid test

Mayer's test. To perform Mayer's test, 0.5 mL of Mayer's reagent was combined with 0.5 mL of the extract and heated briefly. The emergence of a yellow precipitate indicates the presence of alkaloids in the extract. Wagner's test. The plant extract was combined with Wagner's reagent and 0.5 mL of hydrochloric acid, followed by brief heating. The formation of a brown or reddish-colored precipitate indicates the presence of the alkaloids. Dragendroff's test. Dragendroff's reagent was added to 0.5 mL of the plant extracts. A cloudy orange hue will indicate alkaloid presence.

Flavonoid test

Alkaline reagent test. In this test, 0.5 mL of the extract was gradually added to 1 mL of a sodium hydroxide solution until a strong color change was observed. The yellow color should transition to colorless upon the subsequent addition of a diluted hydrochloric acid solution.

Shinoda test. A small amount of magnesium powder was introduced to 0.5 mL of extract, followed by the gradual addition of approximately 0.5 mL of concentrated hydrochloric acid. The appearance of a crimson-red color signifies the existence of flavonoids in the solution.

Saponin test

Foam test. Approximately 0.5 mL of heated distilled water was added to the ethanolic extract of selected plants, which was subsequently cooled and vigorously agitated for 10 seconds. The presence of saponin is indicated by the formation of foam measuring 1-10 cm in height, persisting for 10 minutes without dissipating, even after the addition of 2N hydrochloric acid.

Phenolic/tannin test

Braymer's test. Approximately 3 to 4 drops of ferric chloride solution were added to the ethanolic extract of selected plants and agitated vigorously for 10 seconds. The development of a vivid, deep blue or black hue signifies the existence of tannins or phenolic compounds within the solution.

Steroid/terpenes test

This test was conducted using Salkowki's reagent; 0.5 mL of the extract was combined with an equal volume of Salkowki's reagent. The appearance of a red hue in the upper layer and a yellow hue in the lower layer indicates the existence of terpenes.

Liebermann Burchard's test. In this test, 0.5 mL of the extract was reacted with Liebermann Burchard's reagent. The resulting mixture was examined for the development of a dark pink or red hue or a reddish-brown ring formation. These color changes indicate the presence of sterols in the sample.

Phytochemical quantitative analysis

Total Phenolic Content (TPC)

The total phenolic content (TPC) of the selected plant extracts was determined according to the methodology established by Abeysinghe et al. (2021) using the Folin-Ciocalteu reagent, with minor adjustments. A 0.5 mL volume of appropriately diluted plant extract was mixed with the Folin-Ciocalteu reagent for analysis. Various concentrations of plant extracts from each part were prepared through suitable dilutions based on their total phenolic contents. The absorbance of each solution was measured at a wavelength of 750 nm. A calibration curve was developed utilizing gallic acid as the standard reference compound, with concentrations ranging from 20 µg/mL to 100 µg/mL. The quantification of total phenolic content was expressed in milligrams of gallic acid equivalents per gram of dried extract (mg GAE/g extract).

Total Flavonoid Content (TFC)

The determination of total flavonoid content was conducted utilizing the aluminum chloride method, as per the methodology outlined by Hossen et al. (2022). In summary, 1.0 mL of the extracts were combined with 4.0 mL of distilled water, followed by the introduction of 0.3 mL of a 5% $NaNO_2$ solution. Subsequent to thorough mixing, 0.3 mL of a 10% $AlCl_3$ solution was introduced, and the amalgamation was allowed to settle at ambient temperature for 6 minutes. The volume was then adjusted to 10 mL by the addition of 2 mL of a 1 M NaOH solution and double-distilled water. Following a 15-minute incubation period, the absorbance was gauged at 510 nm utilizing a spectrophotometer. The outcomes were quantified in milligrams of quercetin equivalents per gram of the sample extract.

In vitro anti-diabetic activity

α-glucosidase inhibition assay

The study evaluated the inhibitory potential of certain plant extracts on α-glucosidase activity through a modified chromogenic method, following established protocols by Liu et al. (2016). In this procedure, varying concentrations of the extract (20 µL) were combined with phosphate buffer at pH 6.8, followed by the addition of 50 µL of *α*-glucosidase. After a 10-minute pre-incubation at 37°C, 45 µL of *p*-nitrophenyl glucoside was introduced. Then, 90 µL of 0.1 M Na_2CO_3 solution was added, and the reaction proceeded for 30 minutes at 37°C. The absorbance was then determined at a wavelength of 405 nm. Acarbose and quercetin were used as standard references, and the outcomes were quantified in terms of percentage inhibition (PI)

In vitro anti-tyrosinase activity

The in vitro anti-tyrosinase activity was assessed following the methodology outlined by Khongkarat et al. (2020) with slight adjustments. Different concentrations of the test sample were dissolved in dimethyl sulfoxide (DMSO) to prepare the experimental solutions. The reaction mixture consisted of 120 µL of 2.5 mM L-DOPA in 80 mM phosphate buffer at pH 6.8, 30 µL of phosphate buffer, and 10 µL of the sample solution in DMSO. After thorough mixing, the mixture was pre-incubated at 25°C for 10 minutes. Subsequently, 40 µL of 165 units/mL mushroom tyrosinase in 80 mM phosphate buffer was added, and the reaction was allowed to proceed at 25°C for 5 minutes. Subsequently, the absorbance was assessed at a wavelength of 475 nm employing a microplate reader (Multiskan SkyHigh, Thermo Scientific). Kojic acid served as a reference standard for diphenolase inhibition. Triplicate analyses were conducted for each sample, and the outcomes are expressed as the means±standard error.

Antioxidant properties

DPPH radical scavenging activity

The DPPH free radical was utilized to assess the antioxidant activity of extracts from Baluran National Park, following a method based on Khongkarat et al. (2020) protocol with slight modifications. In brief, 20 µL of various concentrations of selected plant extracts were combined with a 0.1 mM DPPH methanol solution. The reaction mixture was kept in the dark, and after 30 minutes, the absorbance was recorded at 517 nm using a 96-well Thermo Scientific Multiskan SkyHigh RE 6.1.1 microplate reader. The antioxidant activity of the samples was quantified as percentage inhibition (PI) using the formula: %scavenging activity=(Abs control-Abs sample)/Abs control×100. The experiment was performed in triplicate, and the outcomes were presented as the mean±standard deviation of the scavenging activity.

ABTS radical scavenging activity

The antiradical efficacy was assessed using the $ABTS^{\bullet+}$ free radical decolorization assay, as outlined by Sridhar and Charles (2019). Briefly, 20 µL of the extract at varying concentrations was combined with the $ABTS^{\bullet+}$ working solution in a 96-well microplate. Subsequently, the absorbance was measured at 750 nm using spectrophotometry after a 30-minute incubation period. The scavenging potential was determined utilizing a specific formula as described in the DPPH radical scavenging activity section.

Cupric reducing antioxidant capacity (CUPRAC)

The extract's antioxidant potential was evaluated through the CUPRAC (Cupric Ion Reducing Antioxidant Capacity) assay, employing a methodology akin to the one outlined in the literature by Pawlowicz et al. (2021) with minor adjustments. Specifically, 100 µL of extract at varying concentrations was combined with 50 µL of $CuCl_2$ solution, 50 µL of NH_4Ac buffer, and 50 µL of neo-cuproine. After a 30-minute incubation period, the reaction mixture was analyzed spectrophotometrically at 450 nm. The results were evaluated by determining the µmol/g Trolox equivalent antioxidant capacity (TEAC) in comparison to the standard antioxidant compound Trolox, known for its high reduction potential.

Ferric Reducing Antioxidant Power (FRAP)

The effectiveness of selected plant extracts in reducing iron levels was evaluated utilizing a methodology developed by Wairata et al. (2022) with minor adaptation. This

assessment involved the reduction of a colorless iron complex of Fe^{3+}-tripyridyltriazine to a blue complex of Fe^{2+}-tripyridyltriazine. The FRAP reagent was mixed with a prepared solution containing 300 mM acetate buffer, 40 mM HCl, 10 mM TPTZ, and 20 mM $FeCl_3.6H_2O$. Standard calibration curves were established using varying concentrations of $FeSO_4.7H_2O$ with all solutions freshly prepared. Subsequently, 20 µL of the sample solution at different concentrations, 80 µL of distilled water, and the FRAP reagent were combined and incubated at 37°C for 30 minutes. The absorbance was then measured at 593 nm. The difference between the absorbance of the samples and that of the blank was computed to determine the FRAP value and reduction capacity of the extracts. The FRAP value was quantified in µmol/g extract. The experiment was conducted in triplicate.

Data analysis

All data were presented as the mean±standard error of three measurements and analyzed using nonlinear regression analysis. Analysis of Variance (ANOVA) was used to analyze the means of different groups and whether there was any significant difference. The quantitative data obtained were analyzed descriptively. All measurement was carried out in triplicate.

RESULTS AND DISCUSSION

Phytochemicals analysis

Plant chemicals can be categorized into primary and secondary constituents. Primary constituents encompass sugars, proteins, amino acids, purines, pyrimidines found in nucleic acids, and chlorophyll, all of which are vital for plant metabolism. On the other hand, secondary constituents consist of alkaloids, terpenoids, and phenolics, which protect plants against environmental stresses and regulate growth (Busari et al. 2021). A phytochemical analysis was conducted on ethanolic extracts of specific plant species sourced from Baluran National Park (Figure 2, Table 1). This study employed a variety of chemical tests to confirm the existence of secondary compounds, such as alkaloids, flavonoids, saponins, phenolics/tannins, and terpenoids/ steroids. The outcomes of the phytochemical screening are detailed in Table 2.

Table 1. Yield of selected plant extracts from Baluran National Park, East Java, Indonesia based on weight

Code	Selected plants	Local names	Weight of extract (g)	Yield (%, w/w)
BLR1	*Xylocarpus granatum* J.Koenig	*Jomba, niri, nyiri*	57.06	43.58
BLR2	*Pemphis acidula* J.R.Forst. & G.Forst.	*Sentigi, mentigi laut*	13.88	7.15
BLR3	*Ceriops decandra* (Griff.) Ding Hou	*Tingi*	52.63	27.91
BLR4	*Aegiceras corniculata* (L.) Blanco	*Duduk agung, truntung*	26.31	14.05
BLR5	*Bruguiera gymnorrhiza* (L.) Lam.	*Tanjang/lindur*	11.77	6.12
BLR6	*Bruguiera cylindrica* (L.) Blume	*Tanjang, tanjang sukun*	23.12	20.60
BLR7	*Rhizophora stylosa* Griffith	*Tinjang, bakau kecil*	257.26	12.18
BLR8	*Osbornia octodonta* F.Muell.	*Baru-baru atau kayu semilit*	36.45	18.07
BLR9	*Aegiceras floridum* Roem. & Schult.	*Mange-kasihan*	23.71	15.18
BLR10	*Euphorbia atoto* G.Forst.	*Rumput ramukasang*	7.76	17.43
BLR11	*Streblus asper* Lour.	*Sërut, pëlèh*	0.38	0.80
BLR12	*Diospyros maritima* Blume	*Budeng*	35.47	12.95
BLR13	*Trema tomentosa* (Roxb.) H.Hara	*Anggrung*	13.27	10.13
BLR14	*Ardisia humilis* Vahl	*Lempeni, rumpeni*	10.39	8.15
BLR15	*Celtis philippensis* Blanco	*Menjalinan*	73.03	18.10
BLR16	*Strychnos lucida* R.Br.	*Kayu pahit, bidara gunong*	6.31	6.75
BLR17	*Xylocarpus rumphii* (Kostel.) Mabb.	*Nyiri*	27.51	15.18

Table 2. Phytochemicals of ethanol leaves extract of selected plants from Baluran National Park, East Java, Indonesia

Phytochemical test	Selected plants (BLR)																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Alkaloid																	
Mayer's test	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Wagner's test	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Dragendroff's test	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Flavonoid																	
Shinoda's test	-	+	+	-	+	+	+	-	-	+	+	+	+	+	-	+	-
Alkaline reagent test	-	+	+	-	+	+	+	-	-	+	+	+	+	+	-	+	-
Saponin																	
Foam test	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+
Phenolic/tannins																	
Braymer's test	+	+	-	-	+	-	-	-	-	+	+	+	+	+	-	+	-
Triterpenoid/steroid																	
Salkowski's test	-	+	+	-	+	+	-	-	-	+	+	-	+	+	+	+	+
Liebermann Burchard's test	-	+	+	-	+	+	-	-	-	+	+	-	+	+	+	+	+

Notes: (+): Presence, (-): Absence

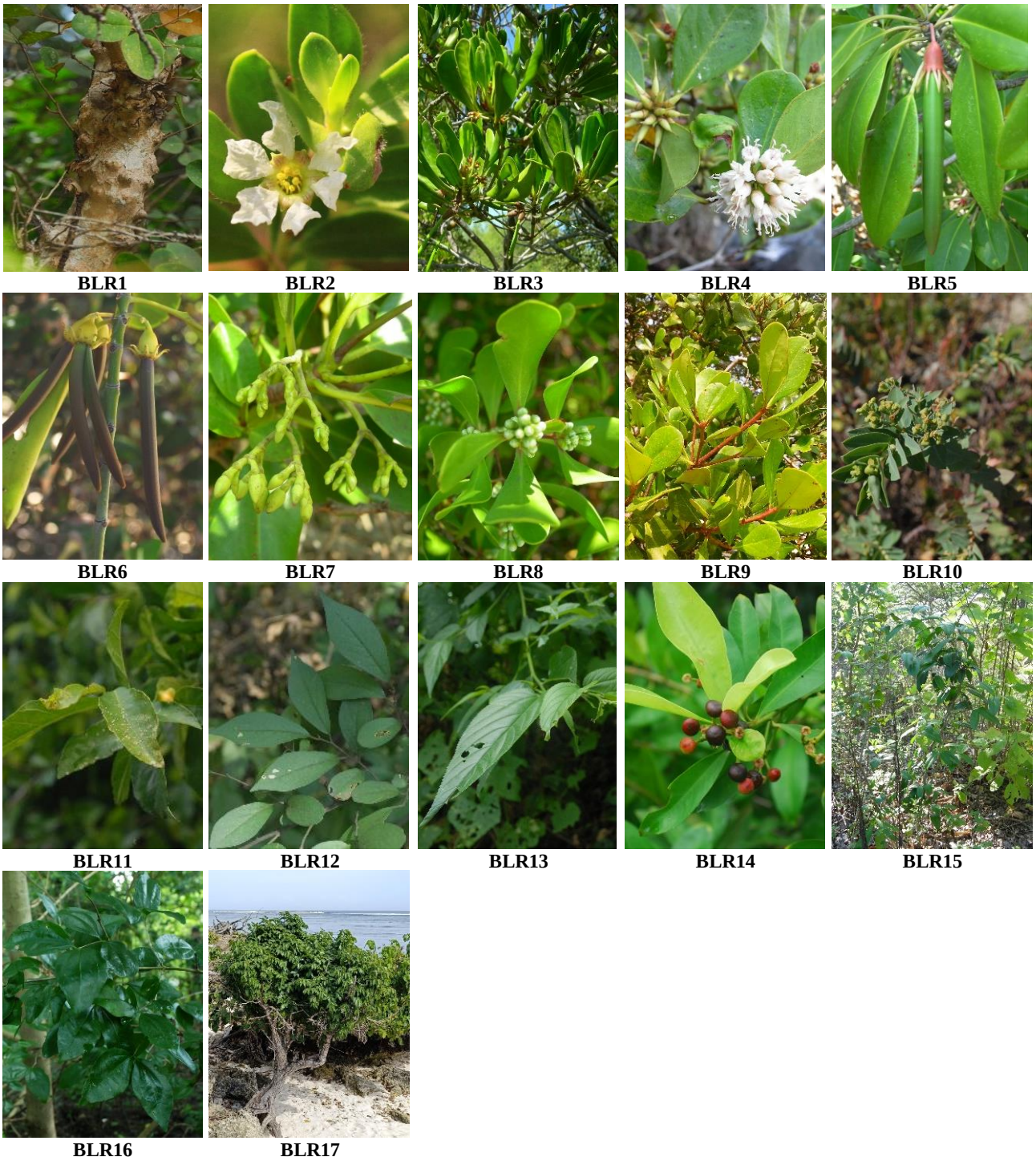

Figure 2. Selected flora from Baluran National Park, East Java, Indonesia

As shown in Table 2, phytochemical screening of ethanol leaf extracts from selected plant species sourced from Baluran National Park showed the presence of phenolics/tannins, flavonoids, alkaloids, triterpenoids/ steroids, and saponins.

The ethanol extracts of *P. acidula*, *E. atoto*, *S. asper*, *T. tomentosa*, and *A. humilis* exhibited the presence of nearly all secondary metabolites except for saponins. The detection of various secondary metabolites in the ethanolic leaf extract of *P. acidula* aligns with the findings of Sundari et al. (2022). In contrast, Wirasisya et al. (2023) identified specific compounds in *E. atoto*, including 24-methylene-cycloartan-3β-ol, jolkinolide E, and tetra-tert-butyl-diphenyl ether [1,10-oxybis(2,4-di-tertbutylbenzene)]. Furthermore,

Pandey and Rastogi (2022) and Ibrahim et al. (2013) documented the presence of various phytochemicals in the leaves of *S. asper*, such as phenolic/tannins, lignans, triterpenoids/steroids, and saponins. A recent study conducted by Chaturvedi (2010) identified a secondary metabolite belonging to the saponin group, specifically 3-O-acetyl-olean-12-ene-28-oic-O-β-D-glucopyranosyl-(1-4)-O-β-D-xylopyranoside. This compound was isolated from the leaves of *S. asper*, a species that grows in India. Additionally, Al-Robai et al. (2022) noted the phenolic and flavonoid content in *T. orientalis*. The identified phytochemical compounds in these extracts are known for their pharmacological activities and medicinal significance. Flavonoids derived from plants exhibit various properties, including anticancer, antiviral, anti-inflammatory, antioxidant, antimicrobial, and anti-diabetic effects (Hassan et al. 2020). Alkaloids, characterized by nitrogen atoms in their chemical structure, can have toxic effects on certain organisms (Barbieri et al. 2017). The phytochemical profile of the selected flora from Baluran National Park, as revealed in this study, demonstrates a diverse array of identified compounds. Furthermore, variations in the composition of individual plants grown in different regions of the world can be attributed to factors such as climate, geographical location, and time of collection (Al-Owamri et al. 2023). The exploration of the phytoconstituents of the selected flora from Baluran National Park and their therapeutic significance represents a compelling and valuable area of study in contemporary times for the treatment of various human ailments with minimal side effects. To the best of the authors' knowledge, this study represents the first report on the phytochemical assessment of ethanol extracts from the leaves of selected flora species from Baluran National Park.

Quantitative phytochemical analysis

Total phenolic content (TPC) and total flavonoid content (TFC)

In this study, the main focus was on quantifying the levels of phenolics and flavonoids creatively and innovatively. Phenolics are renowned for their antioxidant prowess, thanks to their unique redox characteristics that allow them to act as powerful reducing agents, hydrogen donors, and singlet oxygen quenchers (Kumar and Goel 2019). The total phenolic content (TPC) of the ethanolic extracts' is meticulously outlined in Table 3. The hierarchy of total phenolic content within the diverse flora of Baluran National Park, ranging from the least to the most abundant, is as follows: *R. stylosa* (64.66±2.98 mgGAE/g extract)>*O. octodonta* (56.17±2.45 mgGAE/g extract)>*C. decandra* (36.33±1.19 mgGAE/g extract)>*E. atoto* (24.02±2.71 mgGAE/g extract)>*X. granatum* (18.64±1.85 mgGAE/g extract)>*A. floridum* (15.42±1.10 mgGAE/g extract). This ranking is consistent with previous phytochemical investigations highlighting the presence of phenolic and flavonoid compounds in the raw extract. Our findings align with earlier research emphasizing the influence of solvent polarity on the extraction of phenolic compounds (Singh et al. 2017). This discovery is further supported by Suh et al. (2014), who noted a moderate total phenolic content in *R. stylosa*. Additionally, a study on the growth of *C. decandra* in Aceh regions revealed a higher total phenolic content in the leaves at 81.96 mgGAE/g compared to our current results (Indriaty et al. 2023). *Euphorbia* species are renowned for their phenolic compound content; de Araújo et al. (2014) showcased the presence of phenolic compounds in *Euphorbia tirucalli* L., displaying antibacterial properties. Ndam et al. (2016) also identified total phenolic content in *Euphorbia golondrina* L.C.Wheeler, adding to the growing body of knowledge in this field.

Table 3. Phytochemicals quantitative of leave extracts selected plants from Baluran National Park, East Java, Indonesia

Code	Selected plants	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg QE/g extract)
BLR1	*Xylocarpus granatum* J.Koenig	18.64 ± 1.07^{A}	45.81±4.10
BLR2	*Pemphis acidula* J.R.Forst. & G.Forst.	$5.75\pm0.0.18^{F}$	18.06 ± 1.41^{D}
BLR3	*Ceriops decandra* (Griff.) Ding Hou	36.33±0.69	244.08±4.65
BLR4	*Aegiceras corniculata* (L.) Blanco	1.90 ± 0.52^{E}	6.59 ± 1.25^{F}
BLR5	*Bruguiera gymnorrhiza* (L.) Lam.	3.28±0.04	$28.27\pm2.10^{C,E}$
BLR6	*Bruguiera cylindrica* (L.) Blume	4.01±0.16	12.50 ± 2.22^{E}
BLR7	*Rhizophora stylosa* Griffith	64.66±1.72	346.02±4.48
BLR8	*Osbornia octodonta* F.Muell.	56.17±1.42	80.05 ± 2.48^{A}
BLR9	*Aegiceras floridum* Roem. & Schult.	15.42 ± 0.64^{B}	52.42 ± 4.55^{B}
BLR10	*Euphorbia atoto* G.Forst.	24.02±1.57	$33.98\pm1.91^{B,D}$
BLR11	*Streblus asper* Lour.	1.22 ± 0.97^{F}	45.06±2.40
BLR12	*Diospyros maritima* Blume	9.97 ± 0.36^{D}	14.67±1.42
BLR13	*Trema tomentosa* (Roxb.) H.Hara	$6.67\pm1.66^{C,E}$	37.86±3.24
BLR14	*Ardisia humilis* Vahl	15.09 ± 0.19^{A}	113.26±4.71
BLR15	*Celtis philippensis* Blanco	9.45±0.59	46.05 ± 5.79^{C}
BLR16	*Strychnos lucida* R.Br.	$11.48\pm0.90^{B,C}$	77.70 ± 3.86^{A}
BLR17	*Xylocarpus rumphii* (Kostel.) Mabb.	4.95 ± 0.10^{D}	6.19 ± 0.01^{F}

Notes: Values with the same uppercase superscript letter represent that it is not significantly different (P<0.05, one-way ANOVA followed by Bonferroni test). Each value represents the mean±standard error (n=3)

Flavonoids, hydroxylated phenolic compounds, are vital for plant defense, produced in times of trouble like a botanical bat signal. These compounds, such as rutin, quercetin, and apigenin, exhibit significant pharmacological properties, including anti-inflammatory, anti-allergic, liver-saving, germ-fighting, and cancer-battling abilities (Banothu et al. 2017; Tungmunnithum et al. 2018). Furthermore, flavonoid compounds in plants contribute to their antioxidant capabilities through a range of actions, such as interacting with metal ions, neutralizing free radicals, and blocking enzymes that produce an excess of these harmful molecules within the plant system (Seal 2016). Our current investigation reveals a notably elevated concentration of total flavonoids in the ethanolic extract of *R. stylosa*, *C. decandra*, and *A. humilis* Vahl, as detailed in Table 3. Among these species, *R. stylosa* exhibited the highest level of flavonoid compounds (346.02±7.75 mgQE/g), followed by *C. decandra* (244.08±8.06 mgQE/g) and *A. humilis* (113.26±8.16 mgQE/g). These recorded values surpass those reported in previous studies on these species (Khatun et al. 2013; Ahad et al. 2021; Kalasuba et al. 2023). Discrepancies in the outcomes can be attributed to variations in extraction parameters, including the extraction method, duration, solvent type, solvent concentration, and plant component (Sekeroglu et al. 2017). Notably, this research represents the inaugural investigation into the total phenol and flavonoid contents of the selected flora sourced from Baluran National Park, with potential implications for further research and application in the fields of botany, pharmacology, and environmental science.

Antioxidant properties

The capacity of an antioxidant compound to scavenge free radicals in the DPPH assay is directly linked to its ability to donate hydrogen atoms, as indicated by Paula et al. (2021). The study evaluated the antioxidant capacity of ethanolic extracts derived from different plant sources using various methods, including DPPH and ABTS radical scavenging assays, as well as the Cupric Reducing Antioxidant Capacity (CUPRAC) and Ferric Reducing Antioxidant Power (FRAP) tests. This comprehensive approach was necessary as more than a single universal method is required for accurately evaluating the antioxidant properties of plants. Our study specifically focused on the ethanol fraction of selected flora from Baluran National Park, demonstrating its antioxidant capacity through the scavenging of DPPH and ABTS radicals, as detailed in Table 4.

In general, in DPPH and ABTS free radical scavenging assays, a relatively high value of percent inhibitions (PI) is associated with high antioxidant potential. A significant difference was found in the DPPH and ABTS free radical scavenging activity of different plant extracts, where maximum antioxidant activity was observed in the ethanolic plant extracts from Baluran National Park. The antioxidant potential in DPPH assay of the studied species, ranked by PI values, was as follows: *R. stylosa* (83.39±1.37%)>*E. atoto* (82.29±0.34%)>*C. decandra* (77.48±1.44%)>*C. philippensis* Blanco (76.81±1.03%)>*O. octodonta* (71.61±0.41%)>*S. lucida* (66.47±0.79%). The PI values for *R. stylosa* and *E. atoto* do not exhibit a statistically significant difference (p>0.05), nor do the values for *C. decandra* and *C. philippensis* Blanco show a significant difference (p>0.05).

Table 4. Screening of antioxidant activities of selected plants from Baluran National Park, East Java, Indonesia

Code	Selected plants	Percentage inhibition (PI, %)[a]		CUPRAC (TEAC µmol/g)	FRAP (µmol/g)
		DPPH	ABTS		
BLR1	*Xylocarpus granatum* J.Koenig	32.15±0.15^{A}	25.38±0.89^{B}	0.81±0.15	1.05±0.01^{B}
BLR2	*Pemphis acidula* J.R.Forst. & G.Forst.	29.49±00.37	30.98±0.92	1.07±0.18^{C}	1.00±0.02^{B}
BLR3	*Ceriops decandra* (Griff.) Ding Hou	77.48±0.21	80.67±0.20	4.18±0.01^{A}	2.52±0.03
BLR4	*Aegiceras corniculata* (L.) Blanco	18.80±00.48	18.31±0.35^{C}	0.63±0.07	0.83±0.01
BLR5	*Bruguiera gymnorrhiza* (L.) Lam.	12.37±31.80^{C}	25.69±0.70^{B}	0.43±0.01	1.24±0.01^{A}
BLR6	*Bruguiera cylindrica* (L.) Blume	24.50±00.35	37.30±0.11	1.32±0.01	1.12±0.03^{B}
BLR7	*Rhizophora stylosa* Griffith	83.39±0.54^{E}	76.12±0.65	3.65±0.02^{B}	1.79±0.01
BLR8	*Osbornia octodonta* F.Muell.	71.61±0.44	92.61±0.0.10^{A}	4.07±0.12^{A}	2.14±0.02
BLR9	*Aegiceras floridum* Roem. & Schult.	28.43±0.32	43.88±0.60	1.04±0.03^{C}	1.22±0.01^{A}
BLR10	*Euphorbia atoto* G.Forst.	82.29±0.36^{E}	88.87±0.68	1.96±0.03	1.37±0.02
BLR11	*Streblus asper* Lour.	19.56±1.75^{B}	12.29±0.63	0.41±0.01	1.05±0.02^{B}
BLR12	*Diospyros maritima* Blume	11.31±0.69^{B,D}	18.24±0.31^{C}	0.38±0.01^{D}	0.93±0.02^{C}
BLR13	*Trema tomentosa* (Roxb.) H.Hara	9.14±0.88^{C}	12.08±0.31^{D}	0.36±0.01^{D}	0.93±0.01^{C}
BLR14	*Ardisia humilis* Vahl	9.24±0.31^{C}	20.18±0.14	0.51±0.01	0.99±0.02^{D}
BLR15	*Celtis philippensis* Blanco	76.81±0.54	90.19±0.28	3.37±0.03^{B}	1.65±0.01
BLR16	*Strychnos lucida* R.Br.	66.47±0.23	98.66±0.14^{A}	3.31±0.02^{B}	1.94±0.01
BLR17	*Xylocarpus rumphii* (Kostel.) Mabb.	8.16±0.65^{D}	12.11±0.12^{D}	0.42±0.01	0.92±0.01^{D}
Quercetin		96.03±0.01	97.84±0.01		
Ascorbic acid (Vitamin C)		96.88±0.01	98.98±0.01		
BHA (Butylated hydroxyanisole)		96.27±0.01	97.27±0.01		
BHT (Butylated hydroxytoluene)		95.06±0.01	97.01±0.01		
Trolox		96.47±0.01	99.28±0.01		

Note: [a]Inhibitory effects at 100 µg/mL (final concentration). Each value represents the mean±standard error (n=3); [b]Values with the same uppercase superscript letter represent that it is not significantly different (P<0.05, one-way ANOVA followed by Bonferroni test). Each value represents the mean±standard error (n=3)

Our results indicate that the percent inhibitions (PI) values of the methanolic extracts of all the ethanolic plant extracts from Baluran National Park are promising sources of natural antioxidants. Furthermore, quercetin, ascorbic acid (vitamin C), BHA, BHT, and Trolox were used as positive standards. Several studies have previously analyzed the antioxidant potential of selected plants from Baluran National Park, including *C. decandra* (Indriaty et al. 2023), *R. stylosa* (Suh et al. 2014), and *S. lucida* (Sarmento et al. 2015). Samad et al. (2024) reported that the genus *Celtis* contains secondary metabolite flavonoid types such as catechin, naringenin, and apigenin. Zhao et al. (2024) found that *E. atoto* contains trachylobane diterpenoids with anti-inflammatory properties. In addition, a previous study by Wirasisya et al. (2023) reported that Euphobiaceae exhibited anti-bacterial and anti-tumor.

A comprehensive qualitative and quantitative analysis of phytochemicals in selected plant species has demonstrated the presence of flavonoids and phenolic compounds in ethanol extracts. Ethanol has proven to be an effective solvent for extraction, as it facilitates the detection of all four groups of secondary plant metabolites. The phytochemical characteristics of the selected samples exhibited a correlation with their bioactive properties. Previous research has identified these chemical constituents as possessing antioxidant properties (Andriani et al. 2019). Ghasemzadeh et al. (2010) reported that elevated levels of total phenolics and flavonoids correlate with increased antioxidant activity, establishing a linear relationship between the content of these compounds and their antioxidant efficacy. In alignment with the phytochemical properties observed in this study, phenolics and flavonoids emerged as the predominant chemical constituents in the extracts, contributing significantly to their antioxidant activity. Specifically, *R. stylosa* exhibited notable antioxidant activity against DPPH, with a percentage inhibition (PI) value of 83.39±0.54%, and demonstrated comparable antioxidant activity against ABTS, yielding a PI value of 76.12±0.65%. Furthermore, *R. stylosa* displayed commendable CUPRAC and FRAP activities, with values of 3.65±0.02 TEAC µmol/g and 1.79±0.01 µmol/g, respectively. These results strongly indicate a high level of antioxidant activity in the selected plants from National Baluran Park, which is typically associated with the presence of polar phenolic and flavonoid compounds. Additionally, beyond their antioxidant properties, flavonoids and phenolics have been shown to exhibit a range of biological activities, including cytostatic, analgesic, anti-inflammatory, antibacterial, and antioxidant effects (Tungmunnithum et al. 2018). However, this study is the first to report on the biological activities of the ethanolic plant extracts from Baluran National Park against DPPH and ABTS free radicals. It indicates that medicinal plants from the tropical forest in Baluran National Park have the potential as natural antioxidant agents, inspiring and motivating further research in this area. Further in-depth studies are required to analyze and isolate the chemical compounds responsible for their antioxidant activity.

Cupric reducing antioxidant capacity (CUPRAC)

In the evaluation of electron donation activity, a key mechanism of antioxidants, the reduction of Cu^{2+} is commonly employed. In this study, the electron-donating potential of selected plant extracts from Baluran National Park was investigated by assessing their ability to reduce Cu (II). The CUPRAC test utilized copper (II)-neocuproine reagent as the chromogenic oxidant, which is based on the conversion of Cu (II) to Cu (I) by antioxidants present in plant extracts. The reduction activities of the extracts are presented in Table 4, with the results expressed as trolox equivalents using the linear equation of Trolox y=0.009x+0.0548, R2=0.9992. The selected plant extracts from Baluran National Park in this study showed a wide range of CUPRAC values, from 0.36±0.01 to 4.18±0.01 µmol/g TR-equivalent. Table 3 shows TEAC (Trolox Equivalent Antioxidant Capacity) values of selected plant extracts from Baluran National Park in the following order: *C. decandra* (4.18±0.01 µmol/g)>*O. octodonta* (4.07±0.21 µmol/g)>*R. stylosa* (3.65±0.04 µmol/g)>*C. philippensis* Blanco (3.37±0.05 µmol/g)>*S. lucida* (3.31±0.04 µmol/g)>*E. atoto* (1.96±0.05 µmol/g)> *B. cylindrica* (1.32±0.01 µmol/g)>*P. acidula* (1.07±0.31 µmol/g)>*A. floridum* (1.04±0.05 µmol/g). Higher TEAC values indicate a greater reducing antioxidant capacity of selected plant extracts from Baluran National Park. The ability of ethanol extracts to reduce cupric ions through antioxidant mechanisms can be ascribed to the elevated concentration of phenolic compounds, which function as electron donors (Munteanu and Apetrei 2021). The observed activity indicates the existence of reductive compounds, specifically hydrogen donors and electron donors (Chohra et al. 2020). Numerous studies have demonstrated that the reducing properties of polyphenols along with their capacity to form stable complexes with transition metals, particularly iron and copper, can influence various biologically significant processes that involve the redox state of metal ions. The interactions between copper ions and polyphenols, particularly flavonoids, are frequently suggested as a mechanism underlying the antioxidant actions of these natural compounds (Payne et al. 2013). However, the literature does not report the determination of antioxidant power by CUPRAC assays of the selected plant extracts from Baluran National Park. The potential implications of our findings could be significant in the field of antioxidant research.

Ferric reducing antioxidant power (FRAP)

The FRAP assay is utilized to assess the antioxidant capacity by utilizing a combination of ferric ions and tripyridyltriazine (TPTZ) as reactants. During this analysis, antioxidants reduce the ferric ion (Fe(III))-TPTZ complex to ferrous ion (Fe(II))-TPTZ complex, resulting in the development of a distinct blue hue that can be quantified at a wavelength of 593 nm (Chohra et al. 2020). The outcomes of the antioxidant efficacy of selected plant extracts from Baluran National Park are detailed in Table 4, which presents the findings of the Ferric Reducing Antioxidant Power (FRAP) examination. This assay illustrates the presence of electron-donating antioxidants and the transformation of ferric iron (Fe^{3+}) to ferrous ion (Fe^{2+}).

The linear equation representing the ferrous ion (Fe^{2+}) is expressed as y=0.0004x+0.1232, with an R2 value of 0.9971. The selected plant extracts from Baluran National Park exhibited a wide range of FRAP values, spanning from 0.83±0.01 µmol/g to 2.52±0.05 µmol/g. Table 4 shows the FRAP values of selected plant extracts from Baluran National Park in the following order: *C. decandra*>*O. octodonta*>*S. lucida*>*R. stylosa*>*C. philippensis* Blanco>*E. atoto*>*B. gymnorrhiza*>*B. cylindrica*>*A. floridum*>*X. granatum S. asper*>*P. acidula*. The higher the FRAP values (µmol/g Fe/g equivalent) of the selected plant extracts, the greater their reducing antioxidant power. This antioxidant activity indicates the presence of reductive compounds, such as hydrogen and electron donors (Spiegel et al. 2020). Prior research has indicated that polyphenols possess reducing properties and can form stable complexes with transition metals, such as iron and copper, impacting biological processes related to the redox state of metal ions (Fernandes et al. 2016). This study is believed to be the initial exploration of the Ferric-Reducing Antioxidant Power (FRAP) of specific plant extracts sourced from Baluran National Park.

Anti-diabetes and anti-tyrosinase properties

The study aimed to assess the inhibitory properties of seventeen selected plants sourced from Baluran National Park against α-glucosidase and tyrosinase enzymes through in vitro experimentation utilizing *p*-NPG and L-DOPA substrates, as outlined in the methodology section. α-glucosidase is a key enzyme responsible for carbohydrate hydrolysis, crucial for regulating glucose digestion and absorption in the small intestine. In the context of diabetes management, one promising strategy involves the inhibition of carbohydrate-digesting enzymes like α-glucosidase to reduce blood glucose levels and improve metabolic outcomes (Ji et al. 2021; Nipun et al. 2021). The study investigated the inhibitory effects of selected plant extracts obtained from Baluran National Park on *α*-glucosidase activity in vitro, as detailed in Table 5. Among the plant extracts evaluated, *E. atoto* exhibited the most potent *α*-glucosidase inhibitory activity with a percent inhibition (PI) value of 92.07±1.43%. In comparison, acarbose and quercetin exhibited PI values of 25.92±1.61% and 84.79±1.18%, respectively. Zhao et al. (2022) identified various secondary metabolites in *E. atoto*, including seopoletin, kaempferol, *p*-hydroxy-benzaldehyde, aurantiamide acetate, corehoionol C, and 6*β*-hydroxycinnamolide. Additionally, Wirasisya et al. (2023) reported the presence of 24-methylene-cycloartan-3β-ol, jolkinolide E, tetra-tert-butyl-diphenyl ether, *α*-tocopherol, and *β*-sitosterol in the *E. atoto* leaves. These findings suggest that the phytochemical composition of *E. atoto* extract may contribute to its *α*-glucosidase inhibitory activity. Notably, prior research on the α-glucosidase inhibitory potential of *E. atoto* is limited, making this study the first to explore the biological activities of selected plant extracts from Baluran National Park against *α*-glucosidase. The results indicate that selected plants from the tropical forest in Baluran National Park could serve as natural anti-diabetic agents. However, further comprehensive investigations are necessary to identify and isolate the specific chemical compounds responsible for their anti-diabetic properties.

Table 5. Anti-diabetes and anti-tyrosinase activities of selected plants from Baluran National Park, East Java, Indonesia

Code	Selected plants	Percentage inhibition (PI, %)[a]	
		***α*-glucosidase**	**Tyrosinase**
BLR1	*Xylocarpus granatum* J.Koenig	12.03±0.81^{D}	23.96±0.53
BLR2	*Pemphis acidula* J.R.Forst. & G.Forst.	21.42±0.57^{B}	20.29±0.73
BLR3	*Ceriops decandra* (Griff.) Ding Hou	26.58±0.51^{A}	21.01±0.54^{A}
BLR4	*Aegiceras corniculata* (L.) Blanco	13.36±1.27^{D}	21.10±0.12^{A}
BLR5	*Bruguiera gymnorrhiza* (L.) Lam.	9.62±0.28	18.71±0.44^{B}
BLR6	*Bruguiera cylindrica* (L.) Blume	16.52±0.23^{C,D}	18.99±0.17^{B}
BLR7	*Rhizophora stylosa* Griffith	18.97±0.76	20.60±0.64
BLR8	*Osbornia octodonta* F.Muell.	48.60±0.27	22.59±0.47^{C}
BLR9	*Aegiceras floridum* Roem. & Schult.	17.82±1.11^{D}	18.73±0.08^{B}
BLR10	*Euphorbia atoto* G.Forst.	92.07±0.83	25.88±0.51
BLR11	*Streblus asper* Lour.	4.62±1.20	21.58±0.49
BLR12	*Diospyros maritima* Blume	10.05±0.21	22.690.74^{C}
BLR13	*Trema tomentosa* (Roxb.) H.Hara	25.40±0.20^{A}	5.80±0.31^{D}
BLR14	*Ardisia humilis* Vahl	23.73±0.58^{B}	5.43±0.22^{D}
BLR15	*Celtis philippensis* Blanco	11.81±0.46	19.96±0.59^{E}
BLR16	*Strychnos lucida* R.Br.	31.24±0.54	7.99±0.48
BLR17	*Xylocarpus rumphii* (Kostel.) Mabb.	15.02±1.07^{C}	19.27±0.17^{E}
Quercetin		84.79±0.68	ND
Acarbose		25.92±0.93^{A}	ND
Kojic acid		ND	84.26±0.55

Note: [a]Inhibitory effects at 100 µg/mL (final concentration); [b]ND, not determined; [b]Values with the same uppercase superscript letter represent that it is not significantly different (P<0.05, one-way ANOVA followed by Bonferroni test). Each value represents the mean±standard error (n=3)

Additionally, this study meticulously investigated the biological activity of specific plants obtained from Baluran National Park and their impact on tyrosinase. Tyrosinase is an enzyme responsible for catalyzing the *o*-hydroxylation of monophenols and the oxidation of *o*-diphenols to *o*-quinones, ultimately leading to melanin production from L-tyrosine through L-3,4-dihydroxyphenylalanine (L-DOPA) and dopaquinone. The results of the tyrosinase inhibitory assay revealed that certain *E. atoto* extracts at a concentration of 100 µg/mL exhibited higher inhibitory effects on tyrosinase activity with a percent inhibition (PI) value of 25.88±0.88% than other plant extracts. The plant extracts from Baluran National Park displayed varying levels of tyrosinase inhibitory activity, with *E. atoto>X. granatum>D. maritima>O. octodonta>S. asper>A. corniculate>C. decandra>R. stylosa>P. acidula* showing the highest inhibition potency in descending order. The positive control, kojic acid, exhibited a percent inhibition of 84.26±0.96%, surpassing the inhibitory effects of the plant extracts from Baluran National Park. This study represents the first investigation into the inhibitory activity of selected plants from Baluran National Park against various enzymes, including *α*-glucosidase and tyrosinase. It underscores the importance of thorough research in the field of biology and pharmacology.

In conclusion, this research suggests that plant extracts sourced from Baluran National Park exhibit diverse functionalities as anti-diabetic, anti-tyrosinase, and antioxidant agents. Their inhibitory effects on *α*-glucosidase and tyrosinase enzymes, along with their ability to scavenge free radicals such as DPPH, ABTS, CUPRAC, and FRAP, highlight their potential therapeutic benefits. Notably, this investigation represents a significant milestone in the field of ethnobotany, as it is the first ethnobotanical study focusing on the biological activities of selected plant species within this region. The unique contribution of this study lies in its exploration of the potential therapeutic benefits of these selected plant extracts, which hold promise as natural remedies for mitigating hyperglycemia progression linked to oxidative stress associated with degenerative ailments. Furthermore, this study offers valuable insights into the bio-assay-guided isolation of bioactive secondary metabolites from the selected plant extracts in Baluran National Park. Future research should aim to identify the specific constituents within these plant extracts that are responsible for their anti-diabetic and anti-tyrosinase properties, as well as their ability to counteract complications arising from free radicals.

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