Plagiarism Checker X Originality Report

Similarity Found: 15%

Date: Tuesday, February 23, 2021
Statistics: 648 words Plagiarized / 4442 Total words
Remarks: Low Plagiarism Detected - Your Document needs Optional Improvement.

/

INTRODUCTION The evidence from the last several decades strongly supports the development of modern medicine, and there has been an increasing awareness of the importance of medicinal plants. Plants still contribute to health care as prophylaxis and in primary medicine. There are several reasons for using herbs such as easy to obtain, cheaper, safer, efficient, and rarely have side effects1. Plants that have been known to cure disease empirically are an option to examine the current search for new therapeutically effective drugs such as anticancer drugs, antimicrobial drugs, and antioxidants2,3.

Various food research results suggest that phenolic secondary metabolites have significant antioxidant activity. Phenolic compounds have properties to prevent and treat various diseases, including diabetes, cancer, cardiovascular, and neurodegenerative diseases4,5. Zingiberaceae is an important plant family reported with high potential biological activity that can treat various diseases6. Zingiberaceae has a large plant kingdom species in the world, including in Indonesia.

Several new generations were discovered by Cinnamomum, Meistera, and Wurfbainia in the newly discovered Zingiberaceae family. Therefore, it is essential to determine plants' medicinal potency by intensifying studies on medicinal plants7. Previous research Zingiberaceae species such as Etlingera elatior (ginger torch) function as antioxidants8, anticancer9, antibacterial, antifungal, cytotoxic, tyrosinase inhibitory activity10, and immunomodulatory activity11. Literature studies suggest that E. elatior has pharmacological activities such as anti-tumor, anti-hyperglycemic, anti-inflammatory, and anti-hyperuricemic12. Meistera chinensis is one of the species that belong to the Zingiberaceae family.

Meistera chinensis, which is the local plant of Southeast Sulawesi and found in the Konawe Regency. Empirically, it is used as a flavor enhancer in food, aches, and increases body immunity. The safety of potential toxic effects in drug research is vital to ensure their use. The low toxicity and clinical effectiveness of natural compounds are among the researchers' aims to obtain plant biological activity13. Toxicity testing is the first step in drug safety parameters before it becomes a drug product used in humans. One of the toxicity testing methods is Brine Shrimp Lethality Test (BSLT). This method can identify the toxicity of natural ingredients14.

The evidence base of researchers has found that the antioxidants in various plants can be used to treat disease and maintain human health15. In the human body, antioxidant compounds can maintain optimal health. Antioxidants function as anti-carcinogenic, regenerate cells, and anti-mutagenic16. Research on plants and fruits shows strong antioxidants activity such as flavonoids, phenolics, and tannins compounds are

important bioactive which are efficacious for protection from disease.

Consumption of natural antioxidants has been reported to reduce morbidity and mortality from degenerative diseases17,18. Currently, healthcare costs increase with disease progression19. Therefore, from a public health perspective, it is important to know about the recipe and dosage of plants used, especially in terms of toxicity, composition, unique properties for patient protection and safety20. Therefore, this study aims to investigate phytochemical screening, total flavonoid and phenolic content, antioxidant, and in vitro toxicity activity of M. chinensis fruit extract.

MATERIALS AND METHODS Materials Meistera chinensis fruit was obtained from Konawe District, Abuki Village, Southeast Sulawesi. The plant collected was authenticated by The Indonesian Institute of Sciences (LIPI), Indonesia. A voucher specimen (601) of the plant was stored at the Herbarium Bogoriense of The Research Center for Biology, Cibinong Science Center. The fresh fruit material (Figure 1), as well as its simplicia (Figure 2), was subsequently cleaned, then dried at 40°C and protected from the sun for four days, and pulverized with a mechanical grinding machine. / Figure 1. Fresh fruit of M.

chinensis // Figure 2. Simplicia for M. chinensis before (left) and after (right) drying The chemical reagents used include ethanol 95%, n-hexane, silica gel 60 GF254 (Merck), ethyl acetate, $FeCl_3$, NaOH, H_2SO_4, chloroform, Na_2CO_3, HCl, Mayer's reagents, formic acid, acetonitrile, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) (Sigma-Aldrich), potassium persulfate (Merck), ascorbic acid, Trolox (Sigma-Aldrich), Artemia salina, potassium dichromate (Merck), dimethyl sulfoxide, anhydrous acetic acid, gallic acid, quercetin, Folin–Ciocalteu's phenol reagent (Sigma), 25% ammonia 2 N, and Mg powder. Methods Extraction About 3,000 g of M. chinensis fruit powder dissolved with 95% ethanol in a closed glass container for 3 x 24 hours.

The maceration process was carried out for three days and filtered and replaced the solvent. The filtrate was collected and concentrated using a rotary vacuum evaporator at 50°C to obtain a viscous extract of 150 g. Fractionation by vacuum liquid chromatography Separation of chemical compounds was carried out by thin-layer chromatography (TLC) to found suitable eluents as references in vacuum liquid chromatography. Columns made using 60 GF254 silica gel with a ratio of 1:2. Filter paper as a barrier and silica in the column, then added n-hexane to wet the silica.

The solvents n-hexane and ethyl acetate were varied in degree of polarity based on the volume ratio (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 v/v). The vacuum pump was started, and the solvent system was slowly poured into the column. This process was repeated

for all eluent comparisons. Qualitative phytochemicals screening Qualitative phytochemical was carried by standard methods to determine the presence of secondary metabolites compounds of M. chinensis fruit, including alkaloids, terpenoids, flavonoids, phenols and tannins, steroids, and saponins21,22. Alkaloids: About 0.5 g of viscous extract was dissolved with 2 mL of 1% HCl, heated slowly, and filtered. Mayer's Reagent is added to the mixture.

The presence of red sediment indicates alkaloids. Flavonoids: The viscous extract was dissolved with 2 mL of 2% NaOH solution. Flavonoids were indicated by the presence of a viscous yellow color turned colorless with some dilute acids. Phenols and tannins: The viscous extract was dissolved in 2 mL of 2% $FeCl_3$ solution. Phenols and tannins were indicated by greenish-blue or black color. Saponins: The 0.5 g of viscous extract was shaken with 2 mL of distilled water then shaken vigorously. Saponins were indicated by the presence of foam formation. Steroids: The viscous extract was dissolved with 2 mL concentrated H_2SO_4 and chloroform. Steroids were indicated by the formation of red color in the chloroform layer.

Terpenoids: The thick extract was added with 2 mL of chloroform and dryness. About 2 mL of H_2SO_4 were added and heated until 2 minutes. A grayish color indicates terpenoids. Determination of total phenolic contents As much as 10 mg of the extract was dissolved in 10 mL of ethanol. Each pipette was 1 mL of extract solution; then the sample was added with 0.4 mL of Folin–Ciocalteu's reagent, shaken, and left for 4-8 minutes, added with 4 mL 7% Na_2CO_3 solution, and shaken until homogeneous. Distilled water was added to 10 mL and let stand for 2 hours at room temperature.

The absorption was measured at a maximum absorption at 765 nm. The total phenolic content was indicated as mg gallic acid equivalent per 1 g of extract weight (mgGAE/g). The sample measurements were replicated three times23,24. Determination of total flavonoid contents Total flavonoid contents were measured using the colorimetric method with quercetin as standard. About 10 mg of the extract was dissolved in 10 mL of ethanol. As much as 1 mL of the solution was taken and added with 3 mL of methanol, 0.2 mL of 10% $AlCl_3$, 0.2 mL of potassium acetate, and distilled water up to 10 mL. The solution was stored for 30 minutes in a dark place at room temperature.

The absorbance was measured on UV-Vis spectrophotometry expressed at 420 nm. The total flavonoid content was as mg equivalent of quercetin per 1 g of extract weight (mgQE/g). The sample measurements were replicated three times23,24. ABTS's radical scavenging activity Briefly, 2.45 mM $K_2S_2O_8$ solution was dissolved with 7 mM ABTS solution and stored for 24 hours in the darkroom to produce a colored solution containing the ABTS radical cation. Before being used for testing, the ABTS radical

cation was dissolved with 50% methanol, where the initial absorbance was 0.7±0.02 at 745 nm and a temperature of 30°C.

Antioxidant activity was determined by 300 µL of 3 mL of ABTS standard solution in a microcomputer. Absorption reduction is carried out exactly 1-6 minutes after mixing. The percentage of inhibition was determined by the formula24: Scavenging effect (%) = (control absorbance – sample absorbance) control absorbance x 100 Toxicological Evaluation Brine Shrimp Lethality Assay The BSLT method was used to know plant extracts' toxicity test25. The extract was put in a vial containing 5 mL of seawater and 10 Artemia salina leach larvae hatched for 48 hours. The concentrations used in BSLT were 10; 100; 250; 500; 750 µg/mL, and negative control.

The percentage of dead shrimp observed after 24 hours and was calculated. Each concentration had three replications. The toxicity test was determined according to the number of dead larvae. The toxicity test was assessed by determining the LC50 score14,26. % mortality = Total larvae mortality Total larvae x 100 The LC50 score is defined as the concentration of a compound causing 50% mortality of shrimp larvae. Data were analyzed by probit in linear regression y = a + bx. The level of toxicity of a compound was classified according to Meyer et al.25. It was very toxic when the LC50 of =30 µg/mL, moderate toxic with LC50 of >30–1000 µg/mL, and low toxic with LC50 of >1000 µg/mL.

Statistical analysis Total flavonoids and phenolic content were performed by UV-Vis spectrophotometry. Each sample analyzed was replicated three times and was presented as mean (± SD) at least three independent experiments using SPSS version 20.0. Statistical analysis was with ANOVA with statistical significance set at $p < 0.05$. RESULTS AND DISCUSSION Extraction About 3,000 g of plant sample extracted with 22.5 L of 95% ethanol and the vacuous extract obtained was 150 g, and the yield value was 5%—maceration process for 3 x 24 hours. The ethanol extract was then dissolved using ethyl acetate solvent and separated between the soluble and insoluble fractions.

Fractionation by vacuum liquid chromatography The initial fraction with TLC aims to found a solvent system to separate the compound, so its pattern can be seen. Each fractionation process was analyzed with TLC and determined by UV light 254 and 366 nm, then sprayed with CeSO427. Vacuum liquid chromatography separation of the ethyl acetate soluble extract resulted in eight fractions analyzed by TLC using hexane : ethyl acetate (8:2) as eluent. The TLC results show the following chromatograms (Figure 3). / (a) (b) (c) Figure 3.

Chromatogram of fractions M. chinensis fruit extract with UV light 254 nm (a), 366 nm

(b), and cerium sulfate (CeSO4) (c) The chromatogram shows differences in compounds from each fraction as indicated by the difference in the Rf value on the appearance of the spots formed (Figure 3). Visualization of detection of separation results was essential in TLC analysis. The UV detection involves using a UV active compound (indicator) put into a stationary phase.

Shortwave UV light (254 nm) with an indicator (manganese-activated zinc silicate) would produce a light green color, while the compounds would form dark spots. Longwave UV light (366 nm) with an indicator would produce a purple color. Compounds that absorb 366 nm of UV light would appear as different spots, depending on the compound type. Compounds that do not absorb UV light at 254 nm or 366 nm were spray detected using the oxidizing reagent CeSO4 in H2SO4 to form black spots28. The Rf value of the spot appearance of each fraction was presented in the Table I. Table I. The Rf value of M. chinensis fruit fraction No _Fraction _Rf _ _1 _Fraction 1 (F1) _0.96; 0.90; 0.79 _ _2 _Fraction 2 (F2) _0.96; 0.90; 0.79; 0.74; 0.65; 0.53 _ _3 _Fraction 3 (F3) _0.53; 0.40; 0.34; 0.25 _ _4 _Fraction 4 (F4) _0.40; 0.34; 0.25; 0.20 _ _5 _Fraction 5 (F5) _0.00; 0.25; 0.20; 0.10 _ _6 _Fraction 6 (F6) _0.00; 0.10; 0.05 _ _7 _Fraction 7 (F7) _0.05; 0.00 _ _8 _Fraction 8 (F8) _0.00 _ _ The solvent system's determination was carried out using a combination of solvents with low polarity differences to have been mixed.

The solvent mixture of n-hexane and ethyl acetate was a universal eluent system often recommended in chromatography because it was easy to evaporate and adjusts polarity (Table I). n-hexane and ethyl acetate solvents with different degrees of polarity (9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, 4 : 6, 3 : 7, 2 : 8, 1 : 9 v/v) exhibited excellent separation and were suitable for use as a solvent system in separations using column chromatography. Qualitative phytochemicals screening Qualitative phytochemical was performed using colorimetric methods to investigate the M. chinensis fruit extract's secondary metabolites, including terpenoids, saponins, phenolics, steroids, alkaloids, and flavonoids29.

The result presented in Table II shows the presence of phytochemicals. Table II. Phytochemical evaluation of the M. chinensis fruit extract Phytochemical _Reagent _Chemical reaction _Result _ _Terpenoid _H2SO4 and heated _Grayish color _+ _ _Saponin _Distilled water _Stable foam _+ _ _Phenolic _FeCl3 2% _Black coloration _+ _ _Steroid _Liebermann –Burchard reagents _Greenish coloration _+ _ _Alkaloid _Mayer’s reagents _Precipitate _+ _ _Flavonoid _NaOH 2% _Yellow color _+ _ _ Based on Table II, the phytochemical evaluation showed that M.

chinensis fruit extract contains phytochemicals including terpenoids, saponins, phenolics, steroids, alkaloids, and flavonoids. Several secondary metabolites, including

triterpenoids and flavonoids, were potential antioxidant, antibacterial, and toxicity properties. Plants contain various chemical compounds such as alkaloids, phenols, steroids, terpenoids, and flavones were responsible for many pharmacological properties30. Total phenolic contents Quantitative analysis of M.

chinensis fruit's total phenolic content was performed using Folin–Ciocalteu's reagent and analyzed by UV-Vis spectrophotometer. Phenolic contents react with the Folin–Ciocalteu's reagent and form complex blue compounds. The standard solution used was gallic acid, a phenolic compound derived from hydroxybenzoic acid24. The total phenolic contents from extract of M. chinensis fruit could be seen in Table III. Table III. Total phenolic contents from ethanol extract of M. chinensis fruit Replication _Absorbance _Total phenolic (mgGAE/g) _Rate of total phenolic (mgGAE/g) _ _1 _0.324 _29.57 _30.72±1.07 _ _2 _0.328 _31.68 _ _ _3 _0.331 _30.92 _ _ _ Based on Table III, the total phenolic in the ethanol extract of M.

chinensis fruit was 30.72±1.07 mgGAE/g, that each g of the extract was equivalent to 30.72 mg of gallic acid. This study stated that the formation of complexes by phenolic compounds with carbohydrates and proteins, obtained from the solvent methanol in the extraction process compared to other solvents. Secondary metabolites phenolics or polyphenols were very important for plants because they provide antioxidant activity to chelate redox-active metal ions, lipid-free radical chains, and block conversion hydroperoxides to reactive ones' oxyradicals31.

The presence of total phenolic contributes to the antioxidant activity32. The total phenolic in the extract depends on the polarity of the solvent used in the extraction. The high solubility of phenol compounds in polar solvents gives the extract a high concentration33. Total flavonoid contents The M. chinensis fruit ethanol extract's total flavonoid content was determined by the complex formation between aluminum chloride with the keto group on the C-4 atom and the hydroxy group on the C-3 or C-5 atom from the flavone and flavonol groups.

Quercetin was used as a standard for determining flavonoids' levels because quercetin was a flavonol group with a keto group on the C-4 atom and the hydroxyl group on the C-3 and C-5 atoms34. The results of total phenolic contents could be seen in Table IV. Table IV. Total flavonoid contents from ethanol extract of M. chinensis fruit Replication _Absorbance _Total flavonoid (mgQE/g) _Rate of total flavonoid (mgQE/g) _ _1 _0.200 _8.57 _8.02±0.48 _ _2 _0.179 _7.82 _ _ _3 _0.182 _7.68 _ _ _ The total flavonoid content expressed as mg equivalent per g of dry sample was 8.02±0.48 mgQE/g (Table IV). This results in sync with the total phenolic content obtained.

Other research had shown that high phenolic levels also contain large amounts of flavonoids31. Plants were a major source of minerals, vitamins, and other essential nutrients. Most of the medicinal plants contain phenolic acids, flavonoids, and other phytochemicals. In the past, the plants was used as medicine to prevent and treat several diseases31,35. ABTS's radical scavenging activity ABTS radical cation decolorization was an applied approach to determining radical scavenging activities.

It had been studied; the activity of various antioxidant compounds present in plants was determined by the extraction method31. The ethanol extract of M. chinensis was fractioned and obtained fraction 1-8 (F1-F8). The value of inhibition of the fraction at the concentration of 0-50 mg/L showed that M. chinensis had very strong antioxidant activity (F8) and ascorbic acid (AA) as a positive control with IC50 was 42.70±3.53 and 9.58±0,57 mg /L, respectively12 (Figure 4). / Figure 4. ABTS radical scavenging of M. chinensis fruit fraction Present results showed that the ABTS radical scavenging ability can be ranked as F8 > F7 > F6 > F5 > FI> F3 > F2 > F4 (Figure 4).

Based on 50% inhibition concentration, fraction of M. chinensis fruit shows very strong antioxidant activity with IC50 of <50 mg/L (F8), strong category with IC50 of 50-100 mg/L (F1, F5, F6, and F7) and medium category with IC50 of 100-150 mg/L (F2, F3, and F4)36. Plant phenolic compounds had flavonoids that have potent antioxidant activity. Naturally, there were flavonoids in plants that could benefit humans.

Studies on flavonoids had demonstrated various antibacterial, antiviral, anticancer, anti-allergic, and anti-inflammatory activities. Flavonoids were very effective at warding off free radicals, which could cure several diseases5. Findings in other plant products indicate that flavonoids and phenolic acids were the main contributors to antioxidant agents as the IC50 value of free radical activity from various dissolved fractions31. Toxicological Evaluation Brine Shrimp Lethality Assay The toxicity of the M. chinensis fruit extract was measured using the BSLT method. The mortality percentage and LC50 of ethyl acetate fraction of M. chinensis were shown in Table V. Table V.

The LC50 of the M. chinensis fruit Sample _LC50 (mg/L) _ _Ethyl acetate fraction _5.20±0.72 _ _Positive control (potassium dichromate) _2.26±0.60 _ _ The LC50 was a value that indicates the concentration of a toxic compound that causes the death of organisms up to 50%. The toxicity test for larvae mortality (Table V) showed an LC50 value of about 5.20±0.72 mg/L and was included in the highly toxic category (=30 ppm). The LC50 of =30 mg/L is defined as highly toxic; LC50 of =1,000 mg/L as toxic; and LC50 of >1,000 mg/L as non-toxic.

Toxicity testing was a common method that could be used to discover new types of

drugs37. The BSLT method was based on an extract's toxicity test, which was simple, fast, inexpensive, and accurate38. The LC50 toxicity test used a sample of Artemia salina leach larvae at extract concentrations of 0, 10, 100, 250, 500, and 750 ppm (Figure 5). The number of larvae that died was calculated using the SPSS probit analysis. / Figure 5. Ratio of toxicity M. chinensis fruit fraction and positive control (potassium dichromate) to Artemia salina leach larvae The mortality of shrimp larvae by positive control for potassium dichromate was presented as LC50 of 2.26±0.6 mg/L.

This result shows that they were not significantly different from the ethyl acetate fraction on shrimp larvae mortality. The highest mortality rate of Artemia salina larvae at extract concentrations of 250-750 µg/mL was 100% (Figure 5). The extract concentration increased with the death of the shrimp larvae. The larva mortality rate was caused by the concentration of the extract given and the presence of secondary metabolites of the extract (Table II), such as flavonoids, phenolics, and terpenoids.

Zingiberaceae was a widespread plant group in Indonesia and used as herbs with antioxidant, cytotoxic, antibacterial, antifungal, hepatoprotective activities10,39. CONCLUSION This study's results and published data support this conclusion that antioxidants with ABTS radical scavenging activities fraction of M. chinensis fruit show very strong antioxidant activity with an IC50 42.7±3.53 mg/L (F8). The total phenolic and flavonoid contents were 30.72±1.07 mgGAE/g and 8.02±0.48 mgQE/g, respectively. The BSLT toxicity test was found to be very toxic with an IC50 of 5.2±0.72 mg/L. It shows that mainly the fraction of M.

chinensis fruit could be the potential source of natural antioxidants and toxicity agents. ACKNOWLEDGMENT The authors thank the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia for funding Penelitian Kerja Sama Perguruan Tinggi (PKPT) research 2020 No. 231/SP2H/LT/DRPM/2019. REFERENCES Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2013;4:177. doi:10.3389/fphar.2013.00177 Musdalipah, Karmilah. Efektivitas ekstrak daun cabai rawit (Capsicum frutescents L.)

sebagai penumbuh rambut terhadap hewan uji kelinci (Oryctolagus cuniculus). Riset Informasi Kesehatan. 2018;7(1):83-8. doi:10.30644/rik.v7i1.137\ Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv. 2015;33(8):1582-614. doi:10.1016/j.biotechadv.2015.08.001 Cory H, Passarelli S, Szeto J, Tamez M, Mattei J. The Role of Polyphenols in Human Health and Food Systems: A Mini-Review. Front Nutr. 2018;5:87. doi:10.3389/fnut.2018.00087

Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016;5:e47. doi:10.1017/jns.2016.41 Sharifi-Rad M, Varoni EM, Salehi B, Sharifi-Rad J, Matthews KR, Ayatollahi SA, et al. Plants of the Genus Zingiber as a Source of Bioactive Phytochemicals: From Tradition to Pharmacy. Molecules. 2017;22(12):2145. doi:10.3390/molecules22122145 de Boer H, Newman M, Poulsen AD, Droop AJ, Fér T, Hi?n LTT, et al. Convergent morphology in Alpinieae (Zingiberaceae): Recircumscribing Amomum as a monophyletic genus. Taxon. 2018;67(1):6-36. doi:10.12705/671.2 Jackie T, Haleagrahara N, Chakravarthi S.

Antioxidant effects of Etlingera elatior flower extract against lead acetate - induced perturbations in free radical scavenging enzymes and lipid peroxidation in rats. BMC Res Notes. 2011;4:67. doi:10.1186/1756-0500-4-67 Ghasemzadeh A, Jaafar HZE, Rahmat A, Ashkani S. Secondary metabolites constituents and antioxidant, anticancer and antibacterial activities of Etlingera elatior (Jack) R.M.Sm grown in different locations of Malaysia. BMC Complement Altern Med. 2015;15:335. doi:10.1186/s12906-015-0838-6 Chan EWC, Lim YY, Wong SK. Phytochemistry and Pharmacological Properties of Etlingera elatior: A Review. Pharmacogn J. 2011;3(22):6-10. doi:10.5530/pj.2011.22.2

Fristiohady A, Zubaydah WOS, Wahyuni W, Mirda M, Saripuddin S, Andriani R, et al. Immunomodulator Activity of Effervescent Granule of Wualae Fruit (Etlingera elatior (Jack) R.M. Smith) Based on Specific Phagocytic Activity. Borneo J Pharm. 2019;2(2):35-40. doi:10.33084/bjop.v2i2.868 Juwita T, Puspitasari IM, Levita J. Torch Ginger (Etlingera elatior): A Review on its Botanical Aspects, Phytoconstituents and Pharmacological Activities. Pak J Biol Sci. 2018;21(4):151-65. doi:10.3923/pjbs.2018.151.165 Al-Enazi NM. Phytochemical Screening and Biological Activities of Some Species of Alpinia and Convolvulus Plants. Int J Pharmacol. 2018;14(3):301-9. doi:10.3923/ijp.2018.301.309 Saragih G, Tamrin, Marpongahtun, Nasution DY, Abdillah.

Phytochemical screening and toxicity of ethanolic extract of mangrove (Rhizophora mucronata) leaves from Langsa, Aceh Timur. Rasayan J Chem. 2020;13(1):476-80. doi:10.31788/RJC.2020.1315524 Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr J. 2016;15:71. doi:10.1186/s12937-016-0186-5 Anwuchaepe AU, Onyegbule FA, Ajaghaku DL, Nwafor FI, Okoye FBC. Evaluation of the in vivo antioxidant, toxicological and chromatographical profiling of leaf extract and fractions of Crescentia cujete Linn. (Bignoniaceae). Asian Pac J Health Sci. 2017;4(3):43-54. doi:10.21276/apjhs.2017.4.3.8.

Patricia VM, Syaputri FN, Tugon TDA, Mardhatillah A. Antioxidant Properties of Pyrus communis and Pyrus pyrifolia Peel Extracts. Borneo J Pharm. 2020;3(2):64-70.

doi:10.33084/bjop.v3i2.1337 Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines. 2018;5(3):93. doi:10.3390/medicines5030093 Nurhikma E, Wulaisfan R, Musdalipah M. Cost Effectiveness Kombinasi Antihipertensi Candesartan-Bisoprolol dan Candesartan-Amlodipin Pada Pasien Rawat Jalan Penderita Hipertensi. Jurnal Profesi Medika : Jurnal Kedokteran dan Kesehatan. 2019;13(2):54-61. doi:10.33533/jpm.v13i2.1284 Angone SA, Mewono L, Mounanga MB, Medzegue S, Mendene HFE, Ndong JGM, et al. Phytochemical screening and cytotoxicity studies of Chrysophyllum pruniforme Pierre ex Engl. Barks. Pharmacognosy Res. 2013;5(3):195-9. doi:10.4103/0974-8490.112427 Indra I, Nurmalasari N, Kusmiati M. Fenolik Total, Kandungan Flavonoid, dan Aktivitas Antioksidan Ekstrak Etanol Daun Mareme (Glochidion arborescense Blume.). Jurnal Sains Farmasi dan Klinis. 2019;6(3):206-12. doi:10.25077/jsfk.6.3.206-212.2019 Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants. 2017;6(4):42. doi:10.3390/plants6040042 Chandra S, Khan S, Avula B, Lata H, Yang MH, Elsohly MA, et al. Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: a comparative study.

Evid Based Complement Alternat Med. 2014;2014:253875. doi:10.1155/2014/253875 Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complement Altern Med. 2012;12:221. doi:10.1186/1472-6882-12-221 Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 1982;45(5):31-4. doi:10.1055/s-2007-971236 Karchesy YM, Kelsey RG, Constantine G, Karchesy JJ. Biological screening of selected Pacific Northwest forest plants using the brine shrimp (Artemia salina) toxicity bioassay. Springerplus. 2016;5:510. doi:10.1186/s40064-016-2145-1 Fristiohady A, Sadarun B, Wahyuni W, Malaka MH, Ahmad F, Malik F, et al. Isolation and identification of secondary metabolite acetone extract Aaptos sp. and its antioxidant properties and acute toxicity. J Appl Pharm Sci. 2020;10(6):81-9. doi:10.7324/JAPS.2020.10611 Sarker DS, Latif Z, Gray AI, editors. Natural Products Isolation. 2nd Edition (Methods in Biotechnology, Vol. 20). Totowa, NJ.: Humana Press Inc.; 2005. doi:10.1385/1592599559 Erwin E, Pusparohmana WR, Sari IP, Hairani R, Usman U.

Phytochemical and antioxidant activity evaluation of the bark of Tampoi (Baccaurea macrocarpa). F1000Res. 2020;7:1977. doi:10.12688/f1000research.16643.2 Kumar V, Sharma A, Kaur H, Thukral AK, Bhardwaj R. Phytochemical profiling of the leaves of Chenopodium and Polygonum using GC-MS. Res J Pharm Technol. 2015;8(12):1629.

doi:10.5958/0974-360X.2015.00292.9 Esmaeili AK, Taha RM, Mohajer S, Banisalam B. Antioxidant Activity and Total Phenolic and Flavonoid Content of Various Solvent Extracts from In Vivo and In Vitro Grown Trifolium pratense L. (Red Clover). Biomed Res Int. 2015;2015:643285. doi:10.1155/2015/643285 Fidrianny I, Sahar NA, Ruslan KW.

Evaluation of antioxidant activities from various extracts of Dragon fruit peels using DPPH, ABTS assays, and correlation with phenolic, flavonoid, carotenoid content. Int J Res Pharm Sci. 2014;5(2):104-11. Mohsen SM, Ammar ASM. Total phenolic contents and antioxidant activity of corn tassel extracts. Food Chem. 2009;112(3):595-8. doi:10.1016/j.foodchem.2008.06.014 Kamtekar S, Keer V, Patil V. Estimation of Phenolic content, Flavonoid content, Antioxidant and Alpha amylase Inhibitory Activity of Marketed Polyherbal Formulation. J Appl Pharm Sci. 2014;4(9):61-5. doi:10.7324/JAPS.2014.40911 Chaiyasut C, Kesika P, Chaiyasut K, Sittiyuno P, Peerajan S, Sivamaruthi BS. Total Phenolic Content and Free Radical Scavenging Activity of Representative Medicinal Plants of Thailand. Asian J Pharm Clin Res. 2017;10(11):137-41. doi:10.22159/ajpcr.2017.v10i11.20741 Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanarin J Sci Technol. 2004;26(2):211-9. Parasuraman S. Toxicological screening. J Pharmacol Pharmacother. 2011;2(2):74-9. doi:10.4103/0976-500X.81895 Rasyid MI, Yuliani H, Angraeni L. Toxicity Test LC50 of Pineung Nyen Teusalee Seeds (Areca catechu) Extract by Brine Shrimp Lethality Test (BSLT) Methode. IOP Conf Ser Earth Environ Sci. 2020;515:012052. doi:10.1088/1755-1315/515/1/012052 Sahidin S, Wahyuni W, Malaka MH, Jabbar A, Imran I, Manggau MA. Evaluation of Antiradical Scavenger Activity of Extract and Compounds from Etlingera Calophrys Stems. Asian J Pharm Clin Res. 2018;11(2):238-41. doi:10.22159/ajpcr.2018.v11i2.22535

INTERNET SOURCES:

<1% - https://www.mdpi.com/1420-3049/22/1/177/htm
<1% - https://www.researchgate.net/publication/319464899_Phenolic_compounds_Potential_Health_Benefits_and_Toxicity
<1% - https://europepmc.org/article/PMC/PMC7967825
1% - https://www.researchgate.net/publication/228090101_Toxicological_screening
<1% - https://iopscience.iop.org/issue/1755-1315/456/1
<1% - https://www.ospublishers.com/skin-care-creams-formulation-and-use.html
<1% - https://ijppr.com/volume8issue9/
<1% - https://bsapubs.onlinelibrary.wiley.com/doi/full/10.1002/aps3.1167
<1% - https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=9101JR3D.txt
<1% - https://www.phytojournal.com/archives/2017/vol6issue1/PartA/6-1-23-924.pdf

<1% - https://www.sciencedirect.com/science/article/pii/S0301477008611926
<1% -
https://www.researchgate.net/publication/264240727_Preliminary_Phytochemical_Screening_of_Whole_Plant_Extracts_of_Peperomia_pellucida_Linn_HBK_Piperaceae_and_Marsilea_quadrifolia_Linn_Marsileaceae
<1% -
https://iosrjournals.org/iosr-jpbs/papers/Vol11-issue2/Version-3/E1102032834.pdf
<1% -
https://www.researchgate.net/publication/282700302_Pharmacognostic_standardisation_of_Maranta_arundinacea_L_-_An_important_ethnomedicine
<1% - http://www.bioline.org.br/pdf?pr13128
<1% - https://www.tojsat.net/journals/tojsat/volumes/tojsat-volume03-i03.pdf
<1% - https://issuu.com/hasanibrahimkozan/docs/1001_abstractbook_bookofabstracts
<1% - https://globalresearchonline.net/journalcontents/v30-1/28.pdf
<1% -
https://www.slideshare.net/AnnaduraiB/39testicular-enzymes-in-silkworms-eposed-to-high-temperature-and-rm-values-of-bands-in-moths-of-bombyx-mori
<1% -
https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.1079.9880&rep=rep1&type=pdf
<1% -
https://www.researchgate.net/publication/341769732_Bioactivities_of_forest_medicinal_plants_on_kutai_ethnic_Indonesia_of_tapak_leman_Hippobroma_longiflora_L_G_Don
<1% -
https://manualzz.com/doc/34965205/november-2016---prodi-biosain-program-pascasarjana-univer...
<1% -
https://www.researchgate.net/publication/233969697_Studies_on_the_Phytochemical_Properties_and_Brine_Shrimp_Toxicity_of_Essential_Oil_Extracted_from_Myristica_fragrans_Houtt_Nutmeg
<1% - https://www.journalcra.com/archive/201612
<1% - https://www.pnas.org/content/99/18/11599
<1% -
https://www.researchgate.net/publication/332601012_ISOLATION_AND_IDENTIFICATION_OF_CHEMICAL_COMPOUNDS_IN_BUTANOL_FRACTION_OF_POHPOHAN_PILEA_TRINERVIA_ROXB_LEAVES
<1% -
https://www.researchgate.net/publication/338362239_Characterization_of_Secondary_Metabolites_Profile_of_Flavonoid_from_Salam_Leaves_Eugenia_polyantha_Using_TLC_and_UVSpectrophotometry

<1% -
https://doku.pub/documents/handbook-of-thin-layer-chromatography-nk0p5rpxw8l1
<1% -
https://www.researchgate.net/publication/5379405_Green_procedure_with_a_green_solvent_for_fats_and_oils'_determination_-_Microwave-integrated_Soxhlet_using_limonene_followed_by_microwave_Clevenger_distillation
<1% -
https://herbal-medicine.imedpub.com/phytochemical-evaluation-of-various-solvent-extracts-of-the-leaves-fruits-and-shoots-of-ferula-jaeschkeana-vatke.php?aid=17689
<1% -
https://www.academia.edu/21359623/Antioxidant_and_Cytotoxic_Activities_of_Gmelina_arborea_ROXB_Leaves
<1% -
https://content.iospress.com/articles/mediterranean-journal-of-nutrition-and-metabolism/mnm17189
<1% -
https://www.researchgate.net/publication/248510930_Total_phenolic_contents_and_antioxidant_activity_of_corn_tassel_extracts
<1% -
https://www.researchgate.net/publication/225565538_Analysis_of_Flavonoids_in_Portulaca_oleracea_L_by_UV-Vis_Spectrophotometry_with_Comparative_Study_on_Different_Extraction_Technologies
<1% - https://ejournal.undip.ac.id/index.php/ksa/article/view/29653/18025
<1% - https://onlinelibrary.wiley.com/doi/full/10.1111/j.1541-4337.2008.00047.x
<1% - https://www.xiahepublishing.com/2472-0712/ERHM-2020-00069
<1% -
https://www.researchgate.net/publication/305677698_Immune_Cells_Activity_Nitrite_Scavenging_and_ABTS_Radical_Scavenging_Activities_of_Codonopsis_lanceolata_Ethanol_Extracts_from_Districts_in_Korea
<1% -
https://tigerprints.clemson.edu/cgi/viewcontent.cgi?article=2838&context=all_dissertations
<1% -
https://www.researchgate.net/publication/236141184_In_vivo_toxicity_study_of_Lantana_camara
<1% -
https://www.researchgate.net/publication/335900191_Anticancer_activities_of_toxic_isolate_of_Xestospongia_testudinaria_sponge
<1% -
https://www.researchgate.net/publication/280132538_Insecticidal_Activity_of_Ethyl_Acet

ate_Extracts_from_Culture_Filtrates_of_Mangrove_Fungal_Endophytes
<1% -
https://www.researchgate.net/publication/284013802_Impact_of_various_factors_responsible_for_fluctuation_in_plant_secondary_metabolites
<1% - https://www.sciencedirect.com/science/article/pii/S0367326X15300708
<1% -
https://www.researchgate.net/publication/316727014_In_vitro_Cytotoxic_Antioxidant_and_Antimicrobial_Activities_of_Adoninia_merrilli_and_Archontopheonix_tukeri_Fruit_Pericarps
<1% - https://www.alanrevista.org/ediciones/2019/4/art-5/
1% -
https://www.phcogres.com/article.asp?issn=0974-8490;year=2020;volume=12;issue=4;spage=466;epage=470;aulast=Nair
1% - http://www.stuartxchange.org/TorchGinger.html
1% - https://rjptonline.org/AbstractView.aspx?PID=2020-13-10-10
<1% - https://www.japsonline.com/abstract.php?article_id=3190
<1% -
https://www.researchgate.net/publication/337260139_Immunomodulator_Activity_of_Effervescent_Granule_of_Wualae_Fruit_Etlingera_elatior_Jack_RM_Smith_Based_on_Specific_Phagocytic_Activity
<1% -
https://www.researchgate.net/profile/Felix_Nwafor2/publication/319058389_Evaluation_of_the_in_vivo_antioxidant_toxicological_and_chromatographical_profiling_of_leaf_extract_and_fractions_of_Crescentia_cujete_Linn_Bignoniaceae/links/598d4dc0aca272e57ad2c641/Evaluation-of-the-in-vivo-antioxidant-toxicological-and-chromatographical-profiling-of-leaf-extract-and-fractions-of-Crescentia-cujete-Linn-Bignoniaceae.pdf
1% - https://www.journalajacr.com/index.php/AJACR/article/view/30148
1% - http://jsfk.ffarmasi.unand.ac.id/index.php/jsfk/article/view/500
1% - https://www.eurekaselect.com/180718/article
<1% - https://japsonline.com/counter.php?aid=3150
<1% -
https://www.ijpsonline.com/articles/phytochemical-and-antioxidant-analysis-of-the-leaf-extract-of-malaysian-medicinal-plant-iabroma-augustai-l-3443.html
<1% -
https://www.researchgate.net/publication/325595668_Formulation_and_stability_assessment_of_arabica_and_civet_coffee_extracts_based_cosmetic_preparations