Pharmaciana
Vol.14, No.2, July 2024, page. 251-259
ISSN: 2088 4559; e-ISSN: 2477 0256
DOI: 10.12928/pharmaciana.v14i2.27277

Ethanol-based solvent system for recovery antioxidant activity *Centella asiatica* L. Urban and its application in sleep-deprived Rats

Sri Wardatun*, Trirakhma Sofihidayati, Nida Afifah, Juju Juhroh
Pharmacy Program Study, Universitas Pakuan,
Jl. Pakuan,Tegallega, Bogor,West Java, Indonesia

Submitted: 22-09-2023 ***Reviewed: 05-12-2023*** ***Accepted: 29-06-2024***

ABSTRACT

Antioxidants can eliminate free radicals by donating electrons to oxidants. Preclinical and clinical studies show that *Centella asiatica* L. Urban has antioxidant activity. Ethanol was the best solvent for extracting antioxidant compounds from *Centella asiatica* L. Urban. The concentration of ethanol solvents affects the compounds extracted and their antioxidant activities. The study used sleep-deprived rats with glutathione (GSH) concentration parameters to identify ethanol-based solvent systems for the optimum recovery of antioxidant activities and the in vivo antioxidant activity of the most active extract. *C. asiatica* L. Urban powder was macerated with 30, 50, 70, and 96% ethanol, respectively. Using a phosphomolybdate reagent, the extract's antioxidant activity was assessed. In vivo, antioxidant activity was tested on the extract with the highest in vitro antioxidant activity. Ethanol-based solvent systems show different recovery antioxidant activities of *C. asiatica* L. Urban. The antioxidant activity was most recovered in 70% ethanol, with an IC_{50} value of 76.76±25.29 µg/mL. Ethanol 70% *C. asiatica* L. Urban extract given to sleep-deprived rats at 300 mg/kg was not significantly different in glutathione levels from those group rats that received the positive control solution (vitamin C). The *C. asiatica* L. Urban 70% ethanol extract can increase glutathione levels in sleep-deprived rats.

Keywords: *Centella asiatica* L. Urban, antioxidant activity, ethanol, glutathione

Corresponding author:
Sri Wardatun
Pharmacy Program Study, Universitas Pakuan
Jl. Pakuan,Tegallega, Bogor,West Java, Indonesia
Email: sri.wardatun@unpak.ac.id

INTRODUCTION

The plant *Centella asiatica* L. Urban has been utilized for therapeutic purposes for a long time (Gohil et al., 2010). Preclinical and clinical studies show that *C. asiatica* L. Urban has wound healing activity, can be used as a brain stimulant, has cognitive improvement and neuroprotective, antiepileptic, and anticonvulsant activities, antioxidant, anti-gastric ulcer, anti-inflammatory properties, anti-proliferative activity, cardioprotective activity, and antidiabetic activity (Arribas-López et al., 2022; Biswas et al., 2021; Junsi & Siripongvutikorn, 2022; Matthews et al., 2019; Mishra et al., 2022; Ramli et al., 2020; Zweig et al., 2021). There are antiviral, antifungal, and antibacterial properties in *C. asiatica* L. Urban (Biswas et al., 2021). There were 139 secondary metabolites isolated from *C. asiatica* L. Urban (Kunjumon et al., 2022). A compound class of phytochemical constituents has been identified as flavonoids, alkaloids, flavones, glycosides, phenolic acids, saponins, triterpenic acids, sesquiterpenes, monoterpenes, diterpenes, polyacetylenes, amino acids, ketone, sugar, sterols, essential oils, minerals, volatiles, and fatty oils (Biswas et al., 2021). The active ingredients of triterpenoid saponin were asiatic acid, asiaticoside, madekossida, and centelloside (Irham et al., 2019).

The solvent was one of the critical parameters that affected the number of bioactive compounds and activity extracted (Mohapatra et al., 2021; Ng et al., 2020). Solvents also affect cell wall permeability via chemical and physical changes (Oreopoulou et al., 2019). The solvent with different polarities had a significant effect on antioxidant activities. The maceration of *C. asiatica* L. Urban has been carried out using methanol, distilled water, ethyl acetate, and n-hexane as solvents; ethanol has not yet been used (Gunathilake et al., 2019; Ng et al., 2020). The ethanol concentration primarily affects the solvent's polarity and extraction efficiency (Trujillo et al., 2020). Ethanol is a non-toxic organic solvent that is relatively cheap, reusable, environment friendly, and commonly used in the preparation of food or nutraceuticals (Gunathilake et al., 2019; Joshi & Adhikari, 2019). The use of ethanol is expected to increase the extraction efficiency of compounds from *C. asiatica* L. Urban that have antioxidant activity; however, it has been shown that the use of ethanol at high concentrations reduces antioxidant activity (Gunathilake et al., 2019). Thus, it's critical to ascertain how ethanol concentration affects *C. asiatica* L. Urban's antioxidant activities.

Antioxidant activity is defined as the ability to reduce free radicals. Free radicals are highly reactive because their outer electrons are unpaired. Free radicals can impair cells by damaging lipids, proteins, and deoxyribonucleic acid (DNA), which can lead to various chronic and degenerative diseases. Antioxidants can eliminate free radicals; they can donate electrons to oxidants to inhibit their activity (Gulcin, 2020).

This study aimed to extract *C. asiatica* L. Urban used various ethanol concentrations to assess the antioxidant properties of extracts in vitro and in vivo. The phosphomolybdate reagent was utilized to ascertain the in vitro antioxidant activity (Alam et al., 2013). Antioxidant activity in vivo was assessed by quantifying glutathione (GSH) levels. It has been demonstrated that *C. asiatica* L. Urban methanol extract raises GSH levels in vivo; however, 70% ethanol extract has not yet been determined (Arora et al., 2018). GSH in the body has a thiol group or sulfhydryl group derived from cysteine residues, so it can function as an antioxidant capable of lowering nitrogen, reactive oxygen, and electrophiles (Lushchak, 2012).

MATERIALS AND METHOD

Materials

C. asiatica L. Urban was obtained from Bogor, Indonesia, and its identity was determined in the Herbarium Bogoriense, National Research and Innovation Agency, quercetin (Sigma Aldrich, Singapore), ascorbic acid/vitamin C (Merck, Indonesia), and every reagent that was utilized was analytical grade.

Preparation of the extract

The herb was dried, and a powder was prepared. A total of 400 g of dry powder was pretreated with petroleum ether (1:4) for 24 h. The fat-free residue was divided equally by weight and then macerated with 30, 50, 70, or 96% ethanol at 1:10 (v/v), respectively. Then, filtrates were concentrated at 40 °C using a rotary evaporator until a dried extract was obtained. The dried extracts were weighed to obtain yield extract (%), which is determined by weighing the extract in comparison to the weight of *C. asiatica* L. Urban's raw material (Ng et al., 2020).

Preparation of reagents and solutions

To prepare the phosphomolybdate reagent solution, a total of 3.33 mL of sulfuric acid was diluted in 100 mL of aquadest, then 0.4 grams of sodium phosphate and 0.5 grams of ammonium molybdate were added. 30 mM sodium phosphate, 0.6 M sulfuric acid, and 4 mM ammonium molybdate were all present in the reagent solution. Before use, the reagent was always freshly produced (Murtala et al., 2021).

To prepare the quercetin standard solution, 50 mg of quercetin was weighed and dissolved in 50 mL (1,000 µg/mL) of methanol. The following concentrations were then prepared: 50, 60, 70, 80, and 90 µg/mL.

The test solutions were prepared by making stock solutions of extract that had been extracted with 30, 50, 70, and 96% ethanol. The extract was weighed at 50 mg and dissolved in 25 mL of methanol (2,000 µg/mL). Each was then diluted at 600, 400, 200,100, and 100 µg/mL, respectively.

Qualitative in vitro tests of phenolic compound and antioxidant activity

One milliliter of ethanol extract solution was mixed with three drops of a 5% ferric chloride solution. When the extract's color changed to a deep green or bluish-black color, it meant that polyphenolic chemicals were present (Shaikh & Patil, 2020).

To determine the flavonoid components, 1 mL of the extract was heated in 1–2 mL of 50% methanol, added 0.5 g of magnesium powder, and then added with 5–6 drops of chloride acid. Flavones were detected by the formation of an orange-red color and a red color in the extract, respectively (Shaikh & Patil, 2020).

To perform the free radical scavenging test, 0.5 mL of ethanol extract solution and 15 mM DPPH reagent solution were added, respectively. When the samples' color changed from purple to yellow, antioxidant activity was confirmed (Ayoola et al., 2008).

Antioxidant activity test

The antioxidant activity tests were carried out in a test tube after adding one milliliter of phosphomolybdate reagent and one milliliter of extract solution. At 95 ^{0}C, the mixture has been incubated for 60 minutes. The solution was kept at room temperature. Subsequently, 1 mL was taken out of the mixture, and 5 mL of methanol was added. For an optimum incubation time of 30 minutes, the mixture shall be allowed to stand. Using a UV-Vis spectrophotometer (Jasco-V730) at a wavelength of 703.5 nm, the absorbance of the mixture was determined (Alam et al., 2013). The samples were determined to have antioxidant activity when the color changed from yellow to green (Abifarin et al., 2019; Murtala et al., 2021).

In vivo testing

Analysis of glutathione (GSH) levels

Before in vivo testing, a research permit was obtained from the Ethics Commission Faculty of Mathematics and Natural Sciences, Universitas Pakuan, Indonesia, No. 036/KEPHP-UNPAK/11-202. Adult male white Sprague-Dawley rats weighing 180–200 g had been used in this research. The rats were kept in standard cages at 24 ± 1 °C with a 12/12 light/dark cycle, ad libitum for access to water and food. Acclimatization was carried out for one week. Four treatment groups were randomly given to the rats, with six rats in each group. Group 1 was the negative control and was given 1 mL of aquadest.

Group 2 was the positive control and was given ascorbic acid 100 mg/day in 1 mL (Zasowska-Nowak et al., 2021).

Rats in Groups 3 and 4 were given an ethanol extract of *C. asiatica* L. Urban at doses of 300 mg/kg BW and 500 mg/kg BW in 1 mL, respectively (El-Sebaey et al., 2019). After acclimatization, animals were treated with sleep deprivation using gentle handling procedures. This procedure involved brushing fur with a cotton tip applicator, inserting objects into the cage, tapping the cage, and rotating the cage. The test rat group was divided into two batches (Ramanathan et al., 2010). The treatment of each batch was carried out by the same person with the same intensity and duration of treatment. The procedure was carried out for 6 hours (08.00–14.00) for six days and 2 hours on day 7 (08.00–10.00). The handling procedure is carried out for 5 minutes every 30 minutes (Ramanathan et al., 2010; Villafuerte et al., 2015).

One hour after sleep-deprived procedures finished, all group rats were given aquadest, vitamin C solution, or *C. asiatica* L extract, respectively. Administration was performed once (a single dose) using a gastric probe. Two hours after aquadest or ascorbic acid solution or extract has been administered, these animals have been sacrificed. Their livers and kidneys were removed and quickly cut open on ice after being cleaned with previously cold saline. For estimation of GSH content, a mixture of liver (25%) and kidney (10%) homogenates has been prepared and used. Protein in 0.5 mL of mixed tissue homogenate was precipitated with a 20% trichloroacetic acid (125 µL) dilution, incubated for 5 minutes, and then cooled the mixture on ice. After adding 0.6 mL of 5% TCA to the mixture, it was centrifuged at 1,000 rpm for 10 minutes. Next, a 0.2 M phosphate buffer (pH 8.0) solution was mixed with 0.1 mL of the supernatant until 1 mL was obtained. Then 2 mL of freshly made DTNB solution in 0.2 M phosphate buffer was added to supernatant. After 1F0 minutes, the the yellow color of solution was measured using a spectrophotometer at a wavelength of 412 nm (Sheeja & Kuttan, 2006).

Statistical analyses

All measurements were performed in triplicate. The average standard deviation of the results was provided. To assess the importance between groups, statistical analyses have been carried out with ANNOVA. A significance level of 0.05 was chosen for the p-value.

RESULT AND DISCUSSION

The herbal powder was pretreated with petroleum ether to remove fat before being macerated with various concentrations of ethanol. The yield extract is presented in Table 1.

Table 1. Extract yields of *C. asiatica* L. Urban extract using various concentrations of ethanol

Type of solvent Extraction	Yield ± SD (%)
30% ethanol	17.38 ± 1.64
50% ethanol	16.21 ± 0.60
70% ethanol	15.75 ± 0.96
96% ethanol	9.89 ± 1.47

Extraction yields showed the ability of the solvent to obtain a crude extract from dried powders and then use it for further utilization (Azmin & Nor, 2020). Table 1 shows a 30% ethanol extract of *C. asiatica* L. Urban was found to have a higher yield than the others. The increasing extracting yield of *C. asiatica* L. Urban by different concentrations of ethanol solvent was in the following 30% ethanol > 50% ethanol > 70% ethanol > 96% ethanol. The extract shall yield an indicative amount of compounds that have been recovered from the solvent (Oreopoulou et al., 2019; Yeop et al., 2019). The results of extraction yields are proportional to the polarity of the solvent (Ng et al., 2020). The difference in ethanol concentration causes a difference in polarity. The lower concentration of ethanol has a higher polarity because water has a higher polarity than ethanol and can produce a higher extract yield of *C. asiatica* L.

Urban. The solvent will diffuse into plant material during extraction and dissolve compounds according to their polarity (Azmin & Nor, 2020). The 30% ethanol solvent was found to be an efficient solvent for extracting yields. The results are according to Ng et al., 2020, who showed that a solvent with a higher polarity may extract extracts with a higher yield. Generally, the polarity of the solvent can impact to yield of extracts.

The results of preliminary qualitative tests for polyphenols and flavonoids showed that *C. asiatica* L. Urban powder and ethanolic extracts contained polyphenols and flavonoids. The preliminary free radical scavenger test (with the DPPH reagent) and antioxidant activity test (with the phosphomolybdate reagent) showed that powder and ethanol extracts had both antioxidant and free radical scavenging activity. Polyphenols and flavonoids were significant compounds that provided antioxidant activity in *C. asiatica* L. Urban and give a strong correlation to antioxidant activity. The main compounds of polyphenols and flavonoids in *C. asiatica* L. Urban ethanolic extracts are apigenin, rutin, kaempferol, luteolin, quercitrin, quercitin, castilliferol, and chlorogenic acid (Azmin & Nor, 2020; Jhansi & Kola, 2019; Ramli et al., 2020).

Reactive oxidative species (ROS), which cause cell damage, are released when there is an imbalance between oxidants and antioxidants, leading to oxidative stress. ROS can consist of peroxide, hydroxyl ions, and nitric oxide, which form during living metabolism and can cause various diseases like aging, cancer, inflammatory diseases, and cardiovascular diseases. Antioxidants are effective agents to eliminate the action of ROS in the body (Jhansi & Kola, 2019; Ramli et al., 2020). Sources of antioxidant agents can come from herbs, *C. asiatica* L. Urban was one of them (Idris & Mohd Nadzir, 2021). The results of the antioxidant activity assay of *C. asiatica* L. Urban is shown in Table 2. In vitro, antioxidant activity was determined using the phosphomolybdate reagent, which is a mixture of sulfuric acid, ammonium molybdate, and sodium phosphate. The green phosphomolybdate complex is created by the reduction of Mo (VI) to Mo (V) at an acidic pH. This oxidation-reduction reaction is the basis of the phosphomolybdate method (Alam et al., 2013). The 70% ethanol extract was shown to have the strongest antioxidant activity compared to the other extracts. The antioxidant activity of 70% ethanol extract > 96% ethanol extract > 50% ethanol extract > 30% ethanol extract of *C. asiatica* L. Urban (Table 2). The different solvent concentrations show different antioxidant activities. The polarity of the solvent contributed had an impact to the solubility of compounds during extraction. Flavonoids, polyphenol compounds, and other antioxidant agents have different solubility in different ethanol polarities (Azmin & Nor, 2020). This matter causes different ethanol concentrations, resulting in different antioxidant activities. The extract with the highest antioxidant activity was then determined for in vivo testing.

Table 2. Antioxidant activity of *C. asiatica* L. Urban extract which was extracted using various concentrations of ethanol

Type of solvent extraction	IC_{50} ± SD (µg/mL)
quercetin	38.85 ± 13.06
30% ethanol extract	459.74 ± 116.12
50% ethanol extract	364.25 ± 78.13
70% ethanol extract	76.76 ± 25.29
96% ethanol extract	203.95 ± 68.54

Figure 1 shows GSH levels of rats that were treated with positive control solution, negative control solution, or *C. asiatica* L. Urban 70% ethanol extracts with different doses. It can be seen that sleep-deprived rats that received 300 mg/kg of *C. asiatica* L. Urban extract had significantly different GSH levels compared to rats that received a negative control solution (aquadest). In addition, their GSH levels were not significantly different from those of rats that received a positive control solution (oral ascorbic acid).

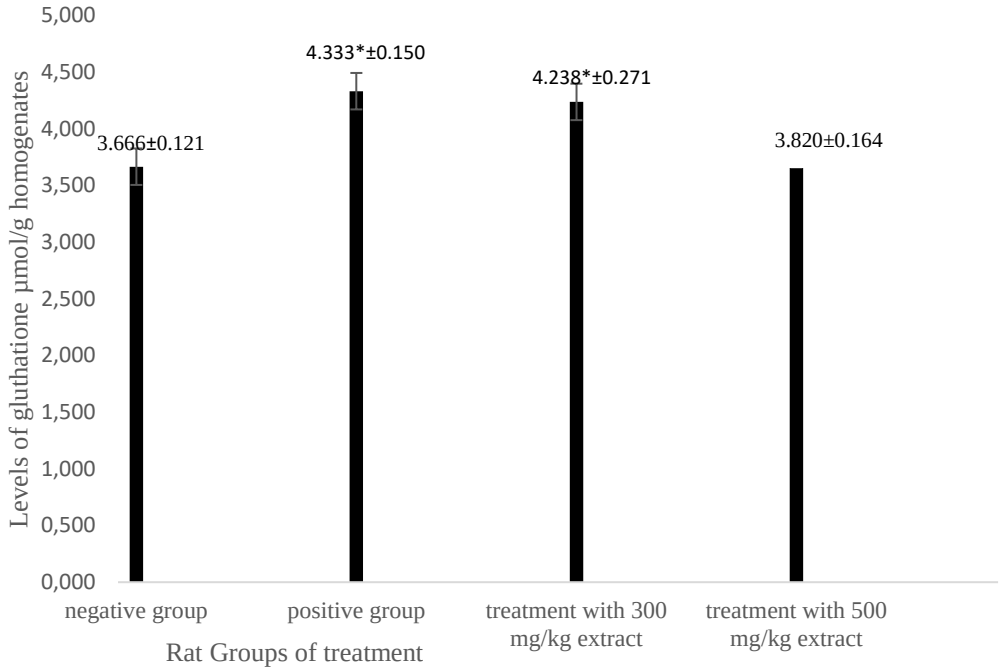

Figure 1. Levels of GSH in a mixture of liver and kidney homogenates of sleep-deprived rats
* Results are significantly different from the negative group ($p < 0.05$)

GSH (glutathione) levels in tissues of treated rats with 70% ethanol extract of *C. asiatica* L. Urban was determined. GSH levels were determined by quantitating the sulfhydryl group (R-SH) present in GSH (Moron et al., 1979). GSH and DTNB react to form the GSH dimer and 2-nitro-5-mercaptobenzoic acid (Appala et al., 2016), a yellow compound (Khan, 2012) that can be detected at 411 nm, which falls within the yellow-green absorption spectrum of 400–435 nm (Shah et al., 2015; Verma & Mishra, 2018). GSH compounds were used as standards in this assay.

Sleep deprivation, or a reduction in sleep time, can promote oxidative stress. When the body's antioxidant systems are overburdened by prooxidants like ROS, oxidative stress results (Villafuerte et al., 2015). Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) as antioxidant enzymes become less active when ROS production surpasses the antioxidant capacity of the cell and induces oxidative stress. This lowers GSH levels and increases ROS accumulation (Gulcin, 2020). Antioxidants are expected to increase reduced GSH levels because oxidative stress can be overcome by the presence of antioxidants. The activity of *C. asiatica* L. Urban extract was traced, and the results are shown in Figure 1. The research demonstrated that giving sleep-deprived rats 300 mg/kg of *C. asiatica* L. Urban extract produced significantly different GSH levels than rats given aquadest (negative control) and similar GSH levels than rats given oral vitamin C (positive control). Vitamin C has antioxidant activity because it can donate electrons to free radicals and can increase GSH levels in the body (Biswas et al., 2021; Gulcin, 2020). GSH levels were not significantly increased by the 500 mg/kg dose of the extract in comparison to the positive control but above the negative control's GSH levels. This is due to the extract's ingredients having saturated the receptors in the body and preventing them from producing the maximum effect (Pasaribu et al., 2012). Therefore, administering the 70% ethanol extract of *C. asiatica* L. Urban which contained antioxidant compounds for sleep-deprived rats, was found to increase GSH levels in the body.

The increased GSH levels detected in rats administered *C. asiatica* L. Urban extracts were related to the overall activity of the natural materials in the extract. Natural materials can induce Nfr2 pathway

signals (Molaei et al., 2021) and play a role in the inactivation of nuclear factor erythroid 2 (Waz et al., 2021). Nrf2 is an important factor in the transcription process that is encoded by the nuclear factor erythroid 2 leucine 2 (NFE2L2) gene and functions to regulate the expression of gene sequences in antioxidant response element (ARE) and influences the synthesis of GSH, GPx, the thioredoxin system, and enzymes related to phase 1 and phase 2 (Molaei et al., 2021). An increase in GSH levels in the body is advantageous because GSH has antioxidant activity; its thiol group or sulfhydryl group derived from cysteine residues allows it to reduce reactive oxygen or nitrogen compounds and electrophiles (Lushchak, 2012).

CONCLUSION

Of all the studied extracts, 70% ethanol extract *C. asiatica* L. Urban's had the highest antioxidant activity. The GSH levels of sleep-deprived rats treated with 300 mg/kg of the extract were found to be comparable to those of rats given vitamin C. Therefore, in sleep-deprived rats, the 70% ethanol extract of *C. asiatica* L. Urban may raise glutathione levels.

ACKNOWLEDGMENTS

All authors give high gratitude to Universitas Pakuan for the financial support of this research under contract number 70. LPPMVII, 2021.

REFERENCES

Abifarin, T. O., Afolayan, A. J., & Otunola, G. A. (2019). Phytochemical and antioxidant activities of Cucumis africanus L.f.: a wild vegetable of South Africa. *Journal of Evidence-Based Integrative Medicine*, *24*, 2515690X1983639. https://doi.org/10.1177/2515690X19836391

Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, *21*(2), 143–152. https://doi.org/10.1016/j.jsps.2012.05.002

Appala, R. N., Chigurupati, S., Appala, R. V. V. S. S., Krishnan Selvarajan, K., & Islam Mohammad, J. (2016). A simple HPLC-UV method for the determination of Glutathione in PC-12 Cells. *Scientifica*, *2016*, 1–6. https://doi.org/10.1155/2016/6897890

Arora, R., Kumar, R., Agarwal, A., Reeta, K. H., & Gupta, Y. K. (2018). Comparison of three different extracts of Centella asiatica for anti-amnesic, antioxidant and anticholinergic activities: in vitro and in vivo study. *Biomedicine & Pharmacotherapy*, *105*, 1344–1352. https://doi.org/10.1016/j.biopha.2018.05.156

Arribas-López, E., Zand, N., Ojo, O., Snowden, M. J., & Kochhar, T. (2022). A systematic review of the effect of Centella asiatica on Wound Healing. *International Journal of Environmental Research and Public Health*, *19*(6), 3266. https://doi.org/10.3390/ijerph19063266

Ayoola, G. A., Folawewo, A. D., Adesegun, S. A., & Abioro, O. O. (2008). Phytochemical and antioxidant screening of some plants of apocynaceae from South West Nigeria. *African Journal of Plant Science*, *2*(9), 124–128.

Azmin, S. N. H. M., & Nor, M. S. M. (2020). Chemical fingerprint of Centella Asiatica's bioactive compounds in the ethanolic and aqueous extracts. *Advances in Biomarker Sciences and Technology*, *2*, 35–44. https://doi.org/10.1016/j.abst.2020.10.001

Biswas, D., Mandal, S., Chatterjee Saha, S., Tudu, C. K., Nandy, S., Batiha, G. E., Shekhawat, M. S., Pandey, D. K., & Dey, A. (2021). Ethnobotany, phytochemistry, pharmacology, and toxicity of Centella asiatica (L.) Urban: A comprehensive review. *Phytotherapy Research*, *35*(12), 6624–6654. https://doi.org/10.1002/ptr.7248

El-Sebaey, A. M., Abdelhamid, F. M., & Abdalla, O. A. (2019). Protective effects of garlic extract against hematological alterations, immunosuppression, hepatic oxidative stress, and renal damage induced by cyclophosphamide in rats. *Environmental Science and Pollution Research*, *26*(15), 15559–15572. https://doi.org/10.1007/s11356-019-04993-7

Gohil, K., Patel, J., & Gajjar, A. (2010). Pharmacological review on Centella asiatica: A potential herbal

cure-all. *Indian Journal of Pharmaceutical Sciences*, *72*(5), 546. https://doi.org/10.4103/0250-474X.78519

Gulcin, İ. (2020). Antioxidants and antioxidant methods: an updated overview. *Archives of Toxicology*, *94*(3), 651–715. https://doi.org/10.1007/s00204-020-02689-3

Gunathilake, K. D. P. P., Ranaweera, K. K. D. S., & Rupasinghe, H. P. V. (2019). Response surface optimization for recovery of polyphenols and carotenoids from leaves of Centella asiatica using an ethanol-based solvent system. *Food Science & Nutrition*, *7*(2), 528–536. https://doi.org/10.1002/fsn3.832

Idris, F. N., & Mohd Nadzir, M. (2021). Comparative studies on different extraction methods of Centella asiatica and extracts bioactive compounds effects on antimicrobial activities. *Antibiotics*, *10*(4), 457. https://doi.org/10.3390/antibiotics10040457

Irham, W. H., Tamrin, Marpaung, L., & Marpongahtun. (2019). Bioactive compounds in Pegagan leaf (Centella asiatica L. Urban) for wound healing. *Journal of Physics: Conference Series*, *1232*(1), 012019. https://doi.org/10.1088/1742-6596/1232/1/012019

Jhansi, D., & Kola, M. (2019). The antioxidant potential of Centella asiatica: a review. *Journal of Medicinal Plants Studies*, *18*(2), 18–20. http://dx.doi.org/10.1016/j.etap.

Joshi, D. R., & Adhikari, N. (2019). An overview on common organic solvents and their toxicity. *Journal of Pharmaceutical Research International*, 1–18. https://doi.org/10.9734/jpri/2019/v28i330203

Junsi, M., & Siripongvutikorn, S. (2022). Development of herbal juice from Centella asiatica: antioxidant property, nutritional value and shelf life of product. *Food Science and Technology*, *42*. https://doi.org/10.1590/fst.93722

Khan, H. (2012). Oxidation of glutathione (GSH) in blood plasma due to oxidative stressors: A case study of silver. *African Journal of Pharmacy and Pharmacology*, *6*(21). https://doi.org/10.5897/AJPP11.790

Kunjumon, R., Johnson, A. J., & Baby, S. (2022). Centella asiatica: Secondary metabolites, biological activities and biomass sources. *Phytomedicine Plus*, *2*(1), 100176. https://doi.org/10.1016/j.phyplu.2021.100176

Lushchak, V. I. (2012). Glutathione homeostasis and functions: potential targets for medical interventions. *Journal of Amino Acids*, *2012*, 1–26. https://doi.org/10.1155/2012/736837\

Matthews, D. G., Caruso, M., Murchison, C. F., Zhu, J. Y., Wright, K. M., Harris, C. J., Gray, N. E., Quinn, J. F., & Soumyanath, A. (2019). Centella asiatica improves memory and promotes antioxidative signaling in 5XFAD mice. *Antioxidants*, *8*(12), 630. https://doi.org/10.3390/antiox8120630

Mishra, A., Singh, Y., Singh, R., Kumar, R., Shukla, S., Kumar, R., Prajapati, Aditi, Rawat, N., Kumar, A., & Pol7, S. L. (2022). Ethano-pharmacology activity & Antioxidant activity of Centella asiatica Plant Parts. *Neuroquantology*, *20*(11), 7562–7572. https://doi.org/10.14704/nq.2022.20.11.NQ66753

Mohapatra, P., Ray, A., Jena, S., Nayak, S., & Mohanty, S. (2021). Influence of extraction methods and solvent system on the chemical composition and antioxidant activity of Centella asiatica L. leaves. *Biocatalysis and Agricultural Biotechnology*, *33*, 101971. https://doi.org/10.1016/j.bcab.2021.101971

Molaei, E., Molaei, A., Abedi, F., Hayes, A. W., & Karimi, G. (2021). Nephroprotective activity of natural products against chemical toxicants: The role of Nrf2/ARE signaling pathway. *Food Science & Nutrition*, *9*(6), 3362–3384. https://doi.org/10.1002/fsn3.2320

Moron, M., Depierre, J., & Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *582*(1), 67–78. https://doi.org/10.1016/0304-4165(79)90289-7

Murtala, R., S. I., Alhassan., H.I, Rasheed., S. N., Dalhatu., S.S, Bala., S.A, Idris., & D. C., Anyanaso. (2021). Comparative phytochemical screening and antioxidant activity of lemon grass and sweet

wormwood leaves extract. *International Journal of Advanced Chemistry*, *9*(1), 20–24. https://doi.org/10.14419/ijac.v9i1.31422

Ng, Z. X., Samsuri, S. N., & Yong, P. H. (2020). The antioxidant index and chemometric analysis of tannin, flavonoid, and total phenolic extracted from medicinal plant foods with the solvents of different polarities. *Journal of Food Processing and Preservation*, *44*(9). https://doi.org/10.1111/jfpp.14680

Oreopoulou, A., Tsimogiannis, D., & Oreopoulou, V. (2019). Extraction of polyphenols from aromatic and medicinal plants: an overview of the methods and the effect of extraction parameters. In *Polyphenols in Plants* (pp. 243–259). Elsevier. https://doi.org/10.1016/B978-0-12-813768-0.00025-6

Pasaribu, F., Sitorus, P., & Bahri, S. (2012). The test of ethanol extract of mangosteen rind (Garcinia mangostana L.) to decrease blood glucose level. *Journal of Pharmaceutics and Pharmacology*, *1*(1), 1–8.

Ramanathan, L., Hu, S., Frautschy, S. A., & Siegel, J. M. (2010). Short-term total sleep deprivation in the rat increases antioxidant responses in multiple brain regions without impairing spontaneous alternation behavior. *Behavioural Brain Research*, *207*(2), 305–309. https://doi.org/10.1016/j.bbr.2009.10.014

Ramli, S., Xian, W. J., & Abd Mutalib, N. A. (2020). A review: antibacterial activities, antioxidant properties and toxicity profile of centella asiatica. *Educatum Journal Of Science, Mathematics And Technology*, *7*(1), 39–47. https://doi.org/10.37134/ejsmt.vol7.1.5.2020

Shah, R. ., Shah, R. ., Pawar, R. ., & Gayakar, P. P. (2015). UV-Visible spectroscopy-review. *International Journal of Institutional Pharmacy and Life Science*, *5*(5), 490–505.

Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603–608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834

Sheeja, K., & Kuttan, G. (2006). Protective effect of andrographis paniculata and andrographolide on Cyclophosphamide-induced urothelial toxicity. *Integrative Cancer Therapies*, *5*(3), 244–251. https://doi.org/10.1177/1534735406291984

Trujillo, A. S. D., Jiménez Forero, J. A., Pineda Insuasti, J. A., González Trujillo, C. A., & García Juarez, M. (2020). Extracción de sustancias bioactivas de Pleurotus ostreatus (Pleurotaceae) por maceración dinámica. *Acta Biológica Colombiana*, *25*(1), 61–74. https://doi.org/10.15446/abc.v25n1.72409

Verma, G., & Mishra, M. (2018). Development and optimization of UV-Vis spectroscopy - a review. *World Journal of Pharmaceutical Research*, *7*(11), 1170–1180.

Villafuerte, G., Miguel-Puga, A., Murillo Rodríguez, E., Machado, S., Manjarrez, E., & Arias-Carrión, O. (2015). Sleep deprivation and oxidative stress in animal models: a systematic review. *Oxidative Medicine and Cellular Longevity*, *2015*, 1–15. https://doi.org/10.1155/2015/234952

Waz, S., Heeba, G. H., Hassanin, S. O., & Abdel-latif, R. G. (2021). Nephroprotective effect of exogenous hydrogen sulfide donor against cyclophosphamide-induced toxicity is mediated by Nrf2/HO-1/NF-κB signaling pathway. *Life Sciences*, *264*, 118630. https://doi.org/10.1016/j.lfs.2020.118630

Yeop, A., Pang, S. F., Law, W. P., Yusoff, M. M., & Gimbun, J. (2019). Assessment of size reduction and extraction methods on the yield of gallic acid from Labisia pumila leaf via Microstructures analysis. *Materials Today: Proceedings*, *19*, 1280–1286. https://doi.org/10.1016/j.matpr.2019.11.134

Zasowska-Nowak, A., Nowak, P. J., & Ciałkowska-Rysz, A. (2021). High-dose vitamin C in advanced-stage cancer patients. *Nutrients*, *13*(3), 735. https://doi.org/10.3390/nu13030735

Zweig, J. A., Brandes, M. S., Brumbach, B. H., Caruso, M., Wright, K. M., Quinn, J. F., Soumyanath, A., & Gray, N. E. (2021). Loss of NRF2 accelerates cognitive decline, exacerbates mitochondrial dysfunction, and is required for the cognitive enhancing effects of Centella asiatica during aging. *Neurobiology of Aging*, *100*, 48–58. https://doi.org/10.1016/j.neurobiolaging.2020.11.019