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CHOLINE CHLORIDE DERIVATIVE-BASED NATURAL DEEP EUTECTIC SOLVENT AS NOVEL GREEN SOLVENT TO EXTRACT COMPONENTS FROM *Spilanthes acmella* STEM

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ABSTRACT

Conventional organic solvents, commonly used for extracting natural compounds, are toxic and pose risks to human health. To address this limitation, green solvent, using natural deep eutectic solvent (NADES), has been developed as an environmentally friendly alternative. Therefore, this study aimed to investigate the use of green solvent NADES with choline chloride and fructose as base materials for extracting active compounds from *Spilanthes acmella* stems. The results of extract analysis conducted through liquid chromatography-mass spectrometry (LCMS) successfully identified 17 active compounds. These compounds included citric acid, sangivamycin, (E)-ferulic acid, methylmalonic acid, lypcosamine, N-acetyl-L-glutaric acid, 6 Hydroxypiconilic acid, pivagabine, and traumatic acid. The extracted active compounds showed pharmacological effects that could be used for pharmaceutical product development.

Keywords: *Spilanthes Acmella*, NADES, Choline Chloride, Green Solvent.

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INTRODUCTION

Spilanthes acmella is a weed species with medicinal compounds, thriving in areas characterized by humid soil and air conditions, specifically, in tropical and subtropical regions.[1] Traditionally, *Spilanthes acmella* flowers are used for treating various diseases, flavoring food, and in the body care industry. All parts of the plant including roots, stems, leaves, and flowers have a bitter taste, suggesting the presence of essential oil. Among several identified compounds, spilanthol[2] has distinct characteristics, with analgesic properties, antioxidant, anti-tumor, anti-inflammatory, antifungal, antibacterial, and immune-stimulating agents.[3-6] The isolation of active compounds in *Spilanthes acmella* is generally carried out using water and other organic solvents.[3,5] However, the organic solvent commonly used is toxic and environmentally unfriendly, suggesting the recent use of environmentally friendly solvents through natural deep eutectic solvents (NADES). Specifically, NADES is an environmentally friendly eutectic solvent derived from natural materials capable of enhancing the extraction capability, stability, solubility, and bioavailability of bioactive compounds in plants. The constituents of NADES are readily available, cost-effective, have low toxicity profiles, and are easy to prepare.[7] In several studies, the development of NADES has been used as an environmentally friendly solvent for extracting bioactive compounds from plants, such as phenols, alkaloids, quercetin, coumarin, and monoterpenes. [8-12] This study aimed to explore the green solvent. NADES with choline chloride and fructose as base materials to extract active compounds from *Spilanthes acmella* stems. The extraction results are analyzed using liquid chromatography-mass spectrometry (LCMS).

EXPERIMENTAL

NADES Preparation

Choline chloride and fructose were used as raw materials for NADES preparation with a molar ratio of 1:2. The materials were weighed, heated, and stirred until the solvent formed, which was used as the extraction medium.

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Extraction Using NADES

NADES (10 mL) was used to extract active compounds from *Spilanthes acmella* stems (1g). This was followed by stirring (65 shaker/minute, 30 minutes) at room temperature. The solution was filtered using filter paper and the extraction results were analyzed for active compound components.

Chemical Composition Analysis Using LCMS

The components in the *Spilanthes acmella* stem extract were analyzed using LCMS.[13]

RESULTS AND DISCUSSION

The analysis of active compound components was conducted on stem extract in choline chloride-fructose-based NADES using LCMS. The results obtained from the analysis are shown in Fig.-1 and Table-1.

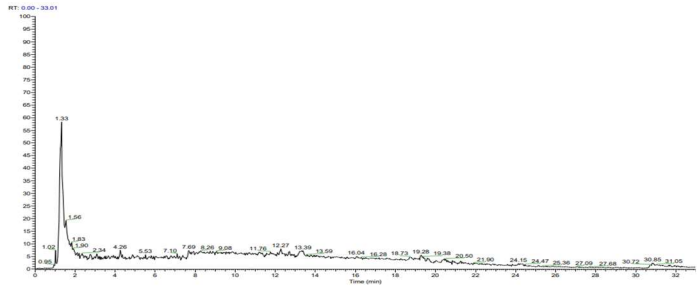

Fig.-1: Spectrum LCMS- *Spilanthes acmella* Stem

Table-1: Active Compounds Extract of *Spilanthes acmella* Stem in NADES

No	Components	Formula	RT
1	Citric acid	$C_6H_8O_7$	1.383
2	4-Oxoproline	$C_5H_7NO_{31}$	1.421
3	Tegaserod	$C_6H_{23}N_{50}$	9.451
4	Sangivamycin	$C_{12}H_{15}N_5O_5$	1.42
5	(E)- Ferulic acid	$C_4H_6O_{41}$	8.988
6	Methylmalonic acid	$C_4H_6O_5$	1.643
7	Aspirin	$C_9H_8O_4$	8.475
8	DL-Malic acid	$C_4H_6O_5$	1.298
9	Lycopsamine	$C_{15}H_{22}NO_5$	9.592
10	Dehydroascorbic acid	$C_6H_6O_6$	1.426
11	(5Z)-9, 12,12,-Trihydroxy-15-octadecenoic acid	$C_{18}H_{34}O_{51}$	3.136
12	6-Hydroxypicolinic acid	$C_6H_5NO_{31}$	1.408
13	Tegaserol	$C_{16}H_{23}N_5O$	10.112
14	N-Acetyl-L-Glutamic acid	$C_7H_{11}NO_{51}$	1.471
15	6-Hydroxy-6-methyl-4,11-dioxoundecanoic acid	$C_{12}H_{20}O_5$	9.8888
16	Pivagabine	$C_9H_{17}NO_3$	8.58
17	Traumatic acid	$C_{12}H_{20}O_{41}$	13.124

Based on the analysis of *Spilanthes acmella* stem extract in choline chloride-fructose-based NADES, 17 compounds with different retention times were identified. The application of NADES in analytical chemistry has proven the ability to extract organic compounds from natural materials. This effectiveness is influenced by intermolecular interactions in the medium, based on hydrogen bonding interactions between NADES and active compounds in the extract.[14]

Among various organic compounds, citric acid has high proportions in fruits such as blueberries, tomatoes, and peaches. However, the high content in lemons, red raspberries, and kiwis can lead to unwanted acidity in processed products,[15] influencing aroma compound formation. For example, pH decreases due to citric acid influences chemical reactions, producing a fruity and sweet aroma.[1]

Sangivamycin is another organic compound known as a potent inhibitor of SARS-CoV-2. This compound is capable of inhibiting live Ebola virus. Additionally, sangivamycin shows broad-spectrum antiviral

activity against Vaccinia virus replication. This efficacy was observed when tested on several cell types,[17] suggesting sangivamycin as a compound with both antibiotic and antiviral activities.[18]

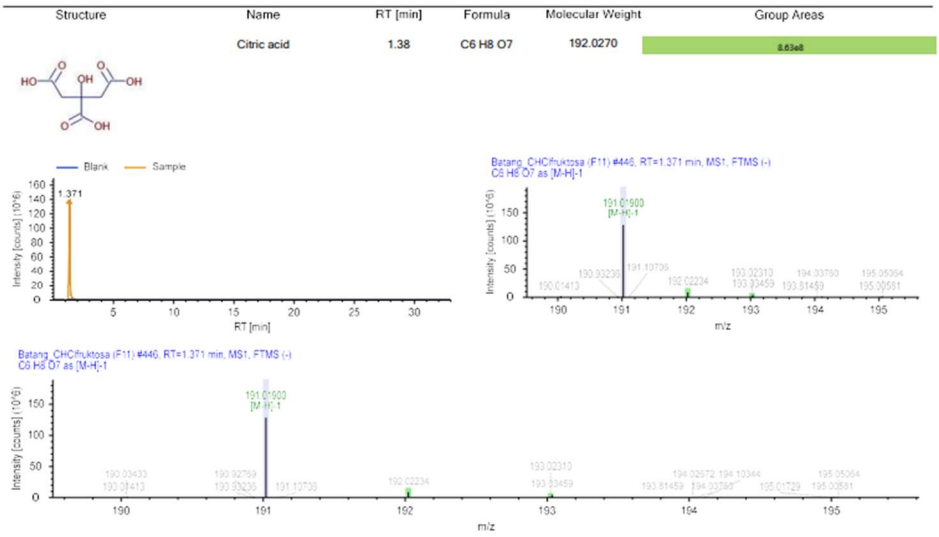

Fig.-2: Mass Spectum of Citric Acid

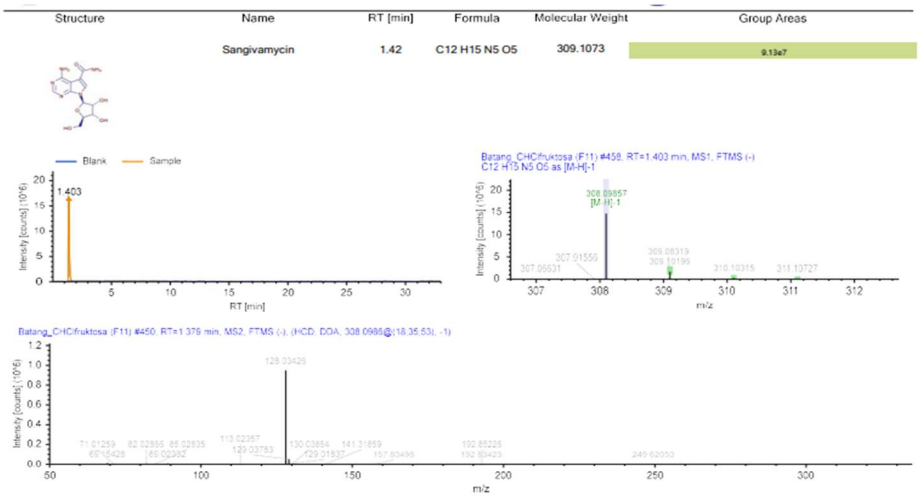

Fig.-3: Mass Spectrum of Sangivamicin

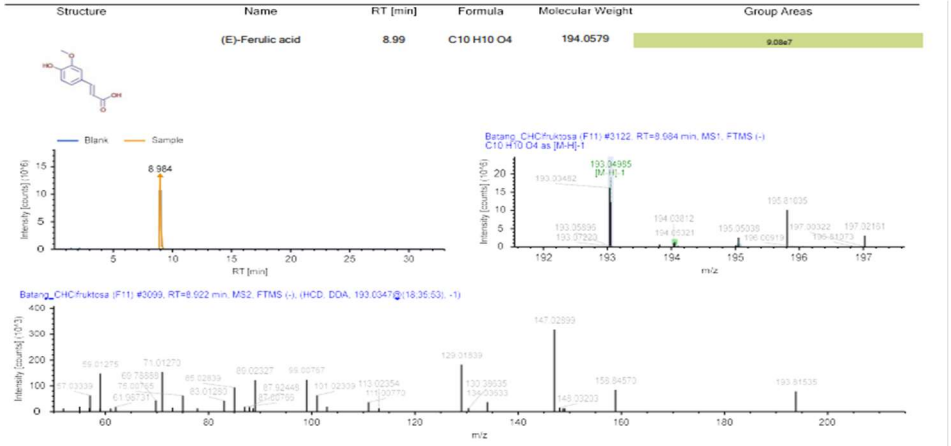

Fig.-4: Mass Spectrum of (E)-Ferulic Acid

Based on the analysis results, ferulic acid was identified in *Spilanthes acmella* stem extract. This compound is found in vegetables, and plant cell walls of monocots, acting as a potent antioxidant. It requires integration into food as a supplement or nutraceutical to alleviate conditions increased by oxidative stress.[19] Ferulic acid is a phenolic compound that possesses anti-inflammatory and antioxidant properties. [20] Previous experimental reports showed that ferulic acid could alleviate brain tissue inflammation and oxidative stress,[21] due to antimicrobial potential and defense responses. Moreover, this study was conducted to evaluate ferulic acid-induced resistance against blue mold in apples and the mechanisms of action.[22]

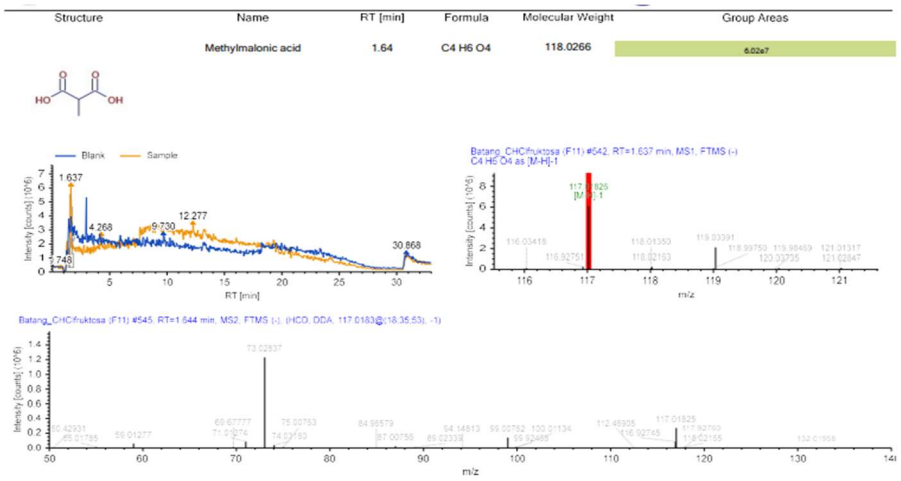

Fig.-5: Mass Spectrum of Methylmalonic Acid

The results of extract analysis conducted through LCMS identified Methylmalonic acid (MMA). Specifically, MMA is the conjugate base of methylmalonate, serving as a dicarboxylic acid found in urine and plasma, and the IUPAC is named Methylpropanedioic acid. Plasma or serum MMA is used in the medical field as a sensitive biomarker for vitamin B-12,[23] particularly in vitamin B12 deficiency.[24] Urinary Methylmalonic acid (uMMA) concentrations can be used as functional markers of vitamin B-12 status. Moreover, uMMA measurement has become an invasive method for screening purposes and epidemiological studies. uMMA is also highly excreted by the kidneys, where the metabolite is concentrated in the urine, serving as a sensitive indicator of tissue depletion.[25]

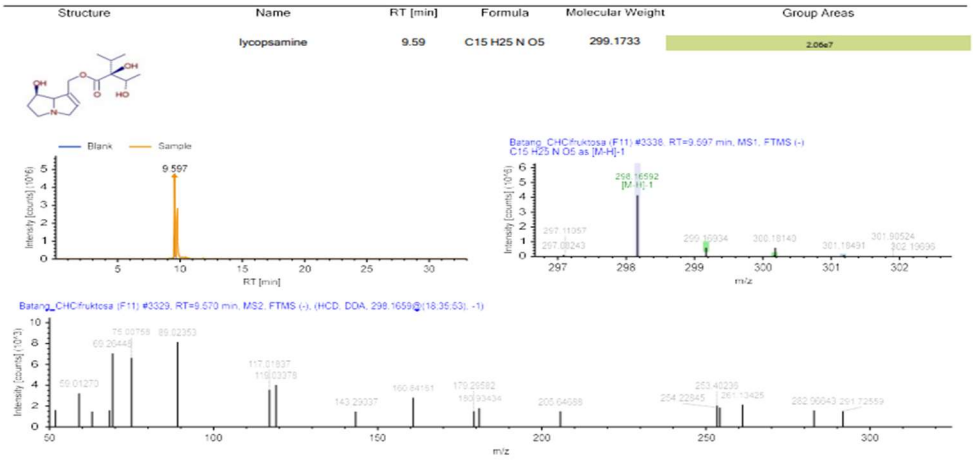

Fig.-6: Mass Spectrum of Lypcosamine

Lycopsamine, widely known as indicine or 9-viridiflorylretronecine, is a natural chemical compound predominantly composed of base nitrogen atoms. Based on pKa, Lycopsamine is water-soluble and a weak acid compound, belonging to the alkaloid group and derivatives. This compound can be found in borage, serving as a potential biomarker for the consumption of food products. Lycopsamine isolated from

Eupatorium maculatum L. has shown potential as an antitumor agent,[26] while *Heliotropium megalanthum* extract shows antifungal effects.[27] This compound is also a potential anti-lung cancer agent and can be a key molecule in lung cancer treatment.[28]

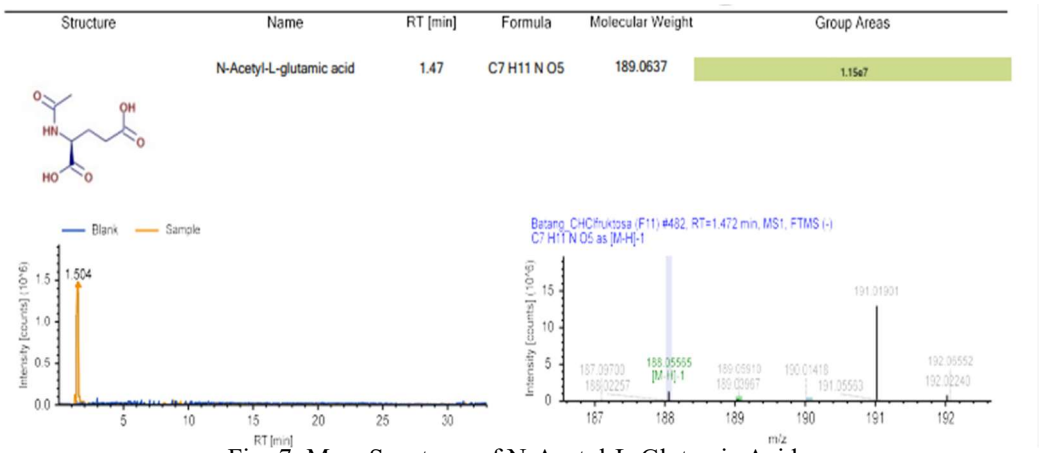

Fig.-7: Mass Spectrum of N-Acetyl-L-Glutamic Acid

N-N-acetyl-L-glutamic acid ($C_7H_{11}NO_5$) identified in this study is an N-acyl-L-amino acid, where one amino hydrogen of glutamic acid is substituted by an acetyl group. This compound can be found in all living species including humans. N-acetyl-L-glutamic acid has been detected in several foods such as jostaberry, and cocoa beans, serving as a potential biomarker for consumption as a food product. Physically, this compound is a solid, white to off-white color used as a flavor enhancer in savory foods. Another compound that was identified included 6-Hydroxypicolinic acid, a derivative of picolinic acid in the pyridine family. This compound is used for the production of 2-oxypyrimidine, which is an important intermediate for drug production.

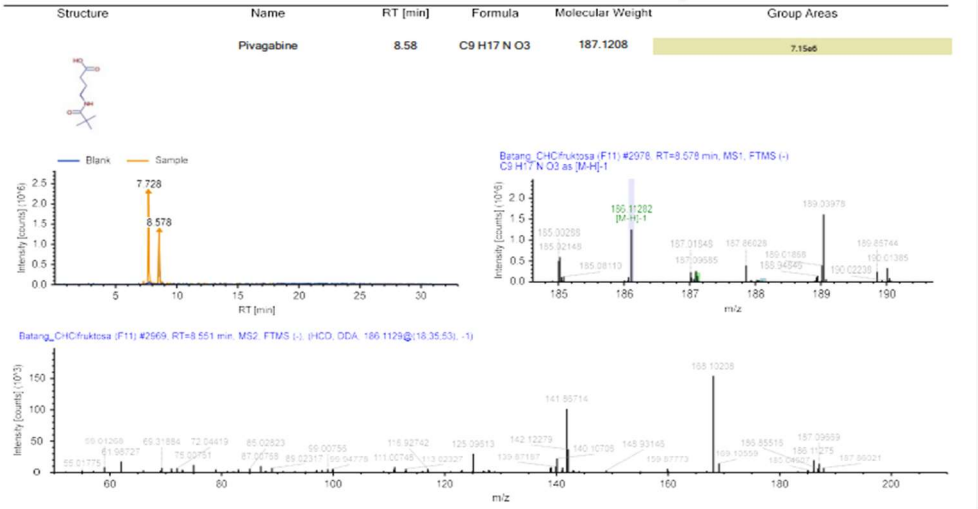

Fig.-8: Mass Spectrum of Pivagabine

Pivagabine is a stable metabolic compound, with biochemical activity in stress conditions (neurosteroid). This compound was used in Italy in 1997 as an antidepressant [29] and tested on experimental rats, proving to prevent deficit behavior associated with stress.[30] Pivagabine has significant therapeutic in patients under psychological pressure due to stressful events.[31]

The analysis identified traumatic acid; a single unsaturated dicarboxylic acid naturally found in plants. Traumatic acid is a wound hormone in plants. It stimulates cell division to form protective calluses. This compound is a potent agent for treating several skin diseases related to oxidative stress and collagen biosynthesis disorders, with potential as an anticancer agent.[32]

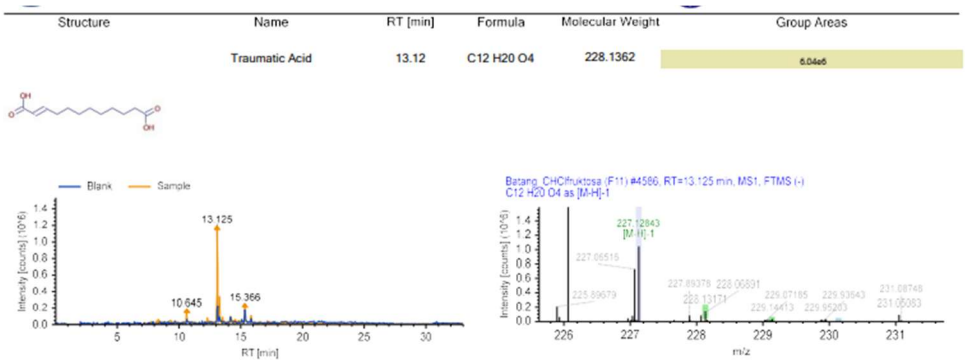

Fig.-9: Mass Spectrum of Traumatic Acid

CONCLUSION

In conclusion, this study showed the successful application of choline chloride and fructose as NADES for the extraction of active compounds from *Spilanthes acmella*. the extraction method is safe for environmentally. Based on the results, the extracted compounds showed potential for application in pharmaceutical, food, and cosmetic industries, suggesting significant potential for further development and application.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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REFERENCES

1. J. Sahu, K. Jain, B. Jain and D.R. Sahu, *Pharmacologyonline,* **2**, 1105(2011).
2. M. Singh and Chatorvedi, *Bioprocess and Biosystems Engineering,* **35**, 943(2012), http://dx.doi.org/10.1007/s00449-012-0679-3
3. K.P.P. Peiris, G.K.J. Silva, W.D. Ratnasouriya, *Journal of Tropical Medicinal Plant,* **2(2)**, 201(2001).
4. B.S. Tanwer, R. Choudhary and R. Vijayvergia, *International Journal of Biotechnology and Biochemistry,* **6(5),** 819(2010)
5. C. Soares, U.R. Lemos, A.G. da Silva, R.M. Campoy, C.A.P. da Silva, R.F. Menegon, I. Rojahn and W.M. Joaquin*, Cell Biology International,* **48**, 131(2014), http://dx.doi.org/10.1002/cbin.10180
6. N. Gupta, A. R. Patel and R. P. Ravindra, *International Journal of Pharma and Bio Science,* **3**,161(2012).
7. K. Owczarek, N. Jatkowska, J. Płotka-Wasylka, M. Rutkowska, O. Shyshchak and M. Bratychak, *Chemistry & Chemical Technology,* **10**, 601(2016), http://dx.doi.org/10.23939/chcht10.04si.601

8. J.B. Barbieri, C. Goltz, C.F. Batistão, T.A. Theodoro, L. Igarashi-Mafra, and M.R. Mafra, *Industrial Crops and Produts,* **144**, 112049(2020), http://dx.doi.org/10.1016/j.indcrop.2019.112049
9. E. Shawky, S.S. Takla, H.M. Hammoda and F.A. Darwish, *Journal of Ethnopharmacology,* **227**, 139(2018), http://dx.doi.org/10.1016/j.jep.2018.08.040
10. Y. Dai Y and K.H. Row, *Molecule,* **24(12)**, 1(2019), http://dx.doi.org/10.3390/molecules24122300
11. W.D. Aryati, A. Nadhira, D. Febianli, F. Fransisca and A, Mun'im, *Journal of Research Pharmacy,* **24(3)**, 389(2020), http://dx.doi.org/10.35333/jrp.2020.161
12. J.M, Jeong, Y Jin, D.E. Yoo, S.Y, Han, E.M. Kim, and J. Lee, *Food Chemistry,* **251**, 69(2018), http://dx.doi.org/10.1016/j.foodchem.2018.01.079
13. A. Maimulyanti, A.R. Prihadi, I, Mapiliandari, *ScieceAsia,* **49**, 515(2023), http://dx.doi.org/10.2306/scienceasia1513-1874.2023.039
14. K, Fraige, R.D. Arrua, A.T. Sutton, C.S. Dunan, A.J. Cavalheiro, E.F. Hilder and V.S. Bolzani, *Journal of Separation Science,* **42**, 591(2019), http://dx.doi.org/10.1002/jssc.201800905
15. J. Mei, X. Liu, X. Liu, Y. Bao, T. Luo and J. Wang, *Journal of Bioscience and Bioengineering,* **136(6)**, 452(2023), http://dx.doi.org/10.1016/j.jbiosc.2023.09.006
16. A. Nishitani, K. Hiramatsu, C. Kadooka, K. Mori, K. Okutsu, Y. Yoshizaki, K. Takamine, K. Tashiro, M. Goto, H. Tamaki and T. Futagami, *Journal of Bioscience and Bioengineering,* **136(6)**, 443(2023), http://dx.doi.org/10.1016/j.jbiosc.2023.09.004
17. R.P. Bennett, Y. Yoluç, J.D. Salter, A. Ripp, H.J. Jessen, S.M. Kaiser, and H.C. Smith, *Antiviral Research*, **218**, 1 (2023), http://dx.doi.org/10.1016/j.antiviral.2023.105716
18. S.H. Krawczyk, M.R. Nassiri, L.S. Kucera, E.R. Kern, R.G. Ptak, L.L. Wotring, J.C. Drach, L.B. Townsend, *Journal of Medicinal Chemistry,* **38,** 4106(1995), http://dx.doi.org/10.1021/jm00020a026
19. N.S. Kumar and H. Nair, *Food and Humanity,* **1,** 1206(2023), http://dx.doi.org/10.1016/j.foohum.2023.09.014
20. R. Batista, Uses and potential applications of ferulic acid, Nova Science Publisher, Inc, New York, USA, p 39(2014)
21. G. Wang, J. Guo, Y. Ma, Y. Xin, X. Ji, Y. Sun, J. Zhang and J. Dong, *Pesticide Biochemistry and Physiology,* **196**, 105590(2023), http://dx.doi.org/10.1016/j.pestbp.2023.105590
22. M. Guo, C. Li, R. Huang, L. Qu, J. Liu, C. Zhang and Y. Ge, *Postharvest Biology Technology,* **202**, 1(2023), http://dx.doi.org/10.1016/j.postharvbio.2023.112378
23. T.H. Schroder, T.A.W. Quay and Y. Lamers, *The Journal of Nutrition,* **144(10)**, 1658(2014), http://dx.doi.org/10.3945/jn.114.194829
24. I.J. Riphagen, I. Minović, D. Groothof, A. Post, M.L.Eggersdorfer, J.E. Kootstra-Ros and S.J.L. Bakker, *BMC Medicine,* **18(1),** 1(2020), http://dx.doi.org/10.1186/s12916-020-01853-x
25. D.B. Matchar, J.R. Feussner and D.S. Millington, *Annals of Internal Medicine,* **106(5),** 707(1987), http://dx.doi.org/10.7326/0003-4819-106-5-707
26. L.H. Zalkow, Glinski, L.T. Gelbaum, T.J. Fleischmann, L.S. Mc Gowan and M.M. Gordon, *Journal of Medicinal Chemistry*, **28(6)**, 687(1985), http://dx.doi.org/10.1021/jm00383a001
27. M. Reina, A. Gonzalez-Coloma, C. Gutierrez, R. Cabrera, J. Henriquez and L. Villarroel, *Journal of Natural Product,* **61,** 1418(1998), http://dx.doi.org/10.1021/np980175a
28. Y. Yue, Q. Huang, Y. Fu and J. Chang, *RSC Advances,* **10,** 23403(2020), http://dx.doi.org/10.1039/D0RA03786A
29. E.V.P. Espinosa, J.P. Murad, F.T. Khasawnen, *Thrombosis*, 173124(2012) http://dx.doi.org/10.1155/2012/173124
30. M. Serra, A. Concas, M.C. Mustallino, M.F. Chessa, M. Stomati, F. Petraglia, A.R. Genazzani and G. Biggio, *Psychoneuroendocrinology*, **24(3)**, 269(1999), http://dx.doi.org/10.1016/S0306-4530(98)00049-3
31. G. Gerra, A. Zaimovic, F. Giusti, M.C. Baroni, R. Delsignore, M.A. Raggi, F. Brambilla, *Behavioural Brain Research* **122(1)**, 93(2001), http://dx.doi.org/10.1016/S0166-4328(01)00177-2
32. A.J. Trypuc, R. Kretowski, E. Wolejko, U. Widro and A. Butarewicz, *Regulatory Toxicity and Pharmacology,* **106**, 137(2019), http://dx.doi.org/10.1016/j.yrtph.2019.04.023

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