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IDENTIFICATION OF COMPOUNDS WITH THERAPEUTIC POTENTIAL FROM ETHANOLIC EXTRACT OF *CENTELLA ASIATICA*

IDENTIFICAREA COMPUȘILOR CU POTENȚIAL TERAPEUTIC DIN EXTRACTUL ETANOLIC DE *CENTELLA ASIATICA*

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ABSTRACT | REZUMAT

Even though the progress made in the field of synthetic and semi-synthetic chemistry in recent years is remarkable, plants still remain an important source of compounds with therapeutic potential. Therefore, recent research has focused on discovering new therapeutic alternatives to classical medication — alternatives that offer increased safety, efficacy, and minimal side effects. Based on the consideration that the pharmacological and therapeutic properties of a species are determined by their chemical components, or the so-called active principles specific to each species, we have proposed a logical approach to a phytotherapeutic study on the plant *Centella asiatica*, starting with the chemical evaluation of the plant and testing its antioxidant activity. The aim of the research was to determine the total content of polyphenols and flavonoids in the ethanolic extract of *Centella asiatica*, as well as to evaluate its antioxidant capacity. The determination of total polyphenols was performed using the Folin -Ciocalteu reagent, while the determination of flavonoids was carried out using the sodium nitrite method, and the evaluation of antioxidant activity was conducted using the DPPH IC50 *method*. Based on the obtained results, we can say that the ethanolic extract of *Centella asiatica* has a high content of polyphenols and flavonoids, demonstrating strong antioxidant activity.

Keywords: *Centella asiatica*, polyphenols, flavonoids, antioxidant activity

Chiar dacă progresele făcute în ultimii ani în domeniul chimiei de sinteză și semisinteză sunt remarcabile, plantele rămân totuși o sursă importantă de compuși cu potențial terapeutic. Astfel, cercetările din ultima perioadă se îndreaptă spre descoperirea unor noi alternative terapeutice față de medicația clasică, alternative care să prezinte siguranță și eficacitate crescută și cât mai puține efecte secundare. Pornind de la considerentul că proprietățile farmacologice și cele terapeutice ale unei specii sunt date de componentele lor chimice sau așa numitele principii active, specifice fiecărei specii, ne-am propus o abordare logică a un studiu fitoterapic asupra plantei *Centella asiatica* începând cu evaluarea chimică a plantei și testarea activității antioxidante. Scopul cercetării a constat în determinare conținutului total de polifenoli și flavonoide din extractul etanolic de *Centella asiatica* precum și evaluarea capacității antioxidante a acestuia. Determinarea polifenolilor totali s-a făcut prin utilizarea reactivului Folin-Ciocâlteu, determinarea flavonoidelor prim metoda cu azotit de sodiu, iar evaluarea activității antioxidante prin *metoda DPPH IC 50.* În urma rezultatelor obținute putem spune că extractul etanolic de *Centella asiatica* are un conținut crescut de plofenoli și flavonoide și prezintă o puternică activitate antioxidantă.

Cuvinte cheie: *Centella asiatica*, polifenoli, flavonoide, activitate antioxidantă

It is native to the tropical regions of Asia, including the Indian subcontinent, Pakistan, Southeast Asia, Malaysia, Indonesia, and India, as well as certain temperate regions of China, Japan, Korea, and Taiwan. It is also found in the equatorial belt of South Africa, Madagascar, and South America. (7, 24).

Centella asiatica is an important medicinal plant, widely used in the East and gaining popularity in the West. The triterpenoids and saponins, the main constituents of *Centella asiatica*, are responsible for its broad therapeutic actions (21, 29).

In nations like Turkey, the cultivation of *Centella asiatica* has proven to be successful, primarily owing to its medicinal significance. This plant boasts a plethora of pharmacological effects that contribute positively to human well-being (23).

While the specialized literature is abundant with research on the plant's pharmacological attributes, its application in diverse animal conditions remains relatively understudied.

Numerous scientific investigations provide evi-

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dence for the analgesic, anticonvulsant, antispasmodic, antidiabetic, antidepressant, anti-inflammatory, antioxidant, antileprotic, anxiolytic, antimicrobial, antitubercular, antifilarial, antipsoriatic, antitumor, antiulcer, immunomodulatory, sedative, stimulant, and wound-healing attributes associated with extracts derived from *Centella asiatica* (1, 4, 8, 13, 16, 18, 19, 25, 27, 28). Historically, *Centella asiatica* extracts have been employed for wound healing purposes, and contemporary research has been steadily bolstering the validity of these assertions. (17).

In a preclinical investigation, it was shown that diverse formulations (including ointment, cream, and gel) incorporating an aqueous extract of *Centella asiatica* and applied to open wounds in rats (administered three times daily for 24 days) led to heightened cellular proliferation and enhanced collagen synthesis at the wound site (16). Furthermore, numerous in vitro and in vivo experiments have shown that the glycosides present in *Centella asiatica* are responsible for its anti-allergic, anti-inflammatory, antioxidant, and antiviral activities (1, 10).

There are authors who have demonstrated the antitumor action of *Centella asiatica* extracts, as they induce apoptosis in human breast adenocarcinoma cells (MCF-7), human colorectal carcinoma cells (Caco), and human cervical cancer cells (HeLa) by inducing nuclear condensation and loss of mitochondrial membrane potential (5, 13).

MATERIALS AND METHODS

The preparation of the extract

To obtain the extract, 10 g of dried and ground *Centella asiatica* were weighed and macerated in 100 ml of 70% (v/v) ethyl alcohol, following the instructions of the Romanian Pharmacopoeia Ed. X (32), for a period of 10 days. The mixture was agitated three times a day and kept in amber bottles. After extraction and pressing the residue, the extracted liquid was allowed to settle at a temperature of 5–10°C and then filtered.

The determination of total polyphenols

For the determination of total polyphenol content, the method described by Tamas-Krumpe Octavia et al. in 2010 (30) was chosen. This procedure involves the use of 0.2 N Folin-Ciocalteu reagent, 7.5% sodium carbonate (Na_2CO_3), and the utilization of gallic acid at various concentrations as a standard.

For the determination, 25 µl of diluted ethanolic extract at a ratio of 1:5 were pipetted (in duplicate) into a 96-well microplate (TPP®). To the sample, 125 µl of 0.2 N Folin-Ciocalteu (FC) reagent and 100 µl of 7.5% Na_2CO_3 were added. The resulting complex was kept at room temperature, protected from light, for 60 minutes, during which a specific blue coloration developed. The optical densities for the analysed extract were quantified spectrophotometrically at a wave length of 765 nm using an Infinite M1000 Pro spectrophotometer (Tecan, Männedorf, Switzerland).

To generate the standard curve, gallic acid was used as the standard at six different concentrations: 0 µg/ml, 15.62 µg/ml, 31.25 µg/ml, 62.50 µg/ml, 125 µg/ml, and 250 µg/ml. The standards were subjected to the same working conditions as the samples (Fig. 1).

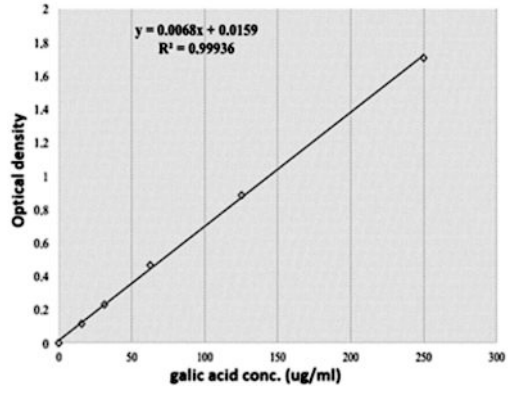

Fig. 1. Gallic acid standard curve

For each extract, the optical density values obtained after subtracting the blank value were inputted into the concentration calculation application using the linear function of the calibration curve.

The determination of flavonoid content using the sodium nitrite method

The determination of total flavonoid content in the ethanolic extract of *Centella asiatica* was carried out following the sequential steps described by Al-Matani et al. in 2015 (2). The extract subjected to the determination was previously diluted with distilled water in a 1:5 ratio, and 500 µl of the prepared sample were pipetted into 5 ml volumetric flasks. To each 500 µl of the sample, 150 µl of freshly prepared $NaNO_2$ (5%) was added, and the resulting mixture was kept for 5 minutes at room temperature.

After 5 minutes, 250 µl of $AlCl_3$ (2%) was pipetted into the mixture, and the reaction time was set to 6 minutes at room temperature. A yellow-coloured complex was formed upon the addition of the aluminium solution. Then, 250 µl of NaOH was added to the newly formed complex, and the reaction time was set to 10 minutes. As a result of this reaction, a reddish-brick colour was obtained, and before the expiration of the 10 minutes, the samples were brought to a final volume of 5 ml with distilled water. The absorbance of the ethanolic extract was read spectrophotometrically at a wavelength of 510 nm using a *Perkin Elmer Lambda 25* spectrophotometer.

Regarding the use of the reference standard, rutin (1 mg/ml) was chosen, and six solutions were pre-

pared with the following concentrations: 0 µg/ml, 62.6 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml, and 1000 µg/ml (Fig. 2).

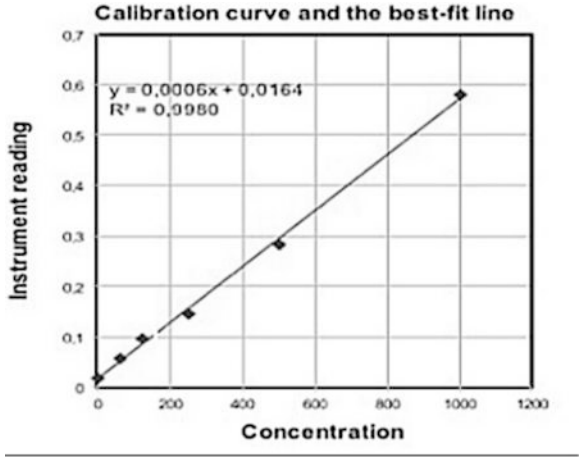

Fig. 2. The standard curve for the six concentrations (0 - 1000 µg/ml) of the standard

The evaluation of antioxidant activity using the DPPH IC50 method

The quantification of the antioxidant activity of the extracts was carried out using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent. In the presence of antioxidants in the extract, the absorbance of DPPH decreases, and the decrease in absorbance is proportional to the antiradical activity. The greater the efficiency of the compound in terms of antiradical activity, the greater the decrease in absorbance.

The degree of decolorization of the DPPH reagent was calculated as the inhibition percentage, which reflects the antioxidant activity of the tested samples. To determine the inhibition percentage, different concentrations of the extract were used, such as 1000, 500, 250, 125, 62.5, 31.25, 15.62, and 7.81 µl/ml, depending on the antioxidant capacity.

The reaction with 2,2-diphenyl-1-picrylhydrazyl (DPPH) took place in microplates by pipetting 25 µl of extract (at each concentration), followed by the addition of 150 µl of DPPH. The optical density was read at a wavelength of 515 nm, and the total reaction time was 30 minutes. Intermediate readings were taken at 5, 10, and 15 minutes during the reaction. The determinations were performed in triplicate, and the degree of decolorization of the DPPH reagent was calculated as the inhibition percentage. This percentage reflects the antioxidant activity of the tested samples, and the two values are directly proportional.

The determinations were carried out within the Research Laboratory for Antioxidant Systems (A.1.c) and the Toxico-pharmacological Research Laboratory (B.12) at the Interdisciplinary Research Platform and the *Horia Cernescu* Research Laboratories Complex, which are infrastructure facilities funded through the RO 05 project. The project is assigned the SMIS-CSNR code 2669 (USAMVBT).

The statistical analysis was performed using the ANOVA (analysis of variance) test and the Bonferroni multiple comparison test using GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

The amount of total polyphenols expressed in gallic acid equivalent for the ethanolic extract of *Centella asiatica* is presented in Table 1, and the amount of flavonoids is presented in Table 2.

Following the results obtained, the number of total polyphenols in the ethanolic extract of *Centella asiatica* was 1033.54 µg/ml and that of flavonoids was 469.42 µg/ml. Based on the obtained results, it can be concluded that the ethanolic extract of *Centella asiatica* has a high content of total polyphenols and flavonoids. Furthermore, there is a direct correlation between the antioxidant capacity of the extract and its high levels of total polyphenols and flavonoids.

Numerous studies have demonstrated that genetic and geographic factors, as well as cultivation, harvesting, and processing practices, all influence the chemical profile of the *Centella asiatica* plant.

Although this has been extensively studied for triterpene compounds, other potentially active components of *C. asiatica*, such as flavonoids and chlorogenic acid derivatives, are also clearly influenced by these factors. These studies emphasise the value of

Table 1

Total polyphenols contained in the ethanolic extracts of *Centella asiatica*

Sample	Replication	Polyphenol content from the calibration curve		
		µg/ml diluted 1:5	µg/ml undiluted	mg/ml undiluted
Ethanolic extract of *Centella asiatica*	replication 1	201.18	1005.91	1.005
	replication 2	209.61	1048.09	1.048
	replication 3	209.32	1046.62	1.046
	Mean of replicates	**206.703**	**1033.54**	**1.033**

Table 2

Determination of flavonoids from *Centella asiatica* ethanolic extract

Sample	Replication	DO (510 nm)	Calculated value (CE) (µg/ml)	Dilution (1:5) (g/l)
Ethanolic extract of *Centella asiatica*	replication 1	0.2790	471.45	2.3572
	replication 2	0.2773	468.53	2.3426
	replication 3	0.2772	468.27	2.3413
	Mean of replicates		**469.42**	**2.3471**

Table 3

The average for IC 50 in the two extracts tested

Sample	IC 50 (Expressed in µL extract/ml)*[4]				
	Replication 1	Replication 2	Replication 3	Mean	SD
Ethanolic extract of *Centella asiatica*	46.44	45.21	46.32	**45.99**	**0.68**

analysing the chemical properties of the *Centella asiatica*. The presence of various bioactive phenolic compounds and carotenoids, such as triterpene saponins, asiaticoside, numerous derivatives of caffeic acid, and flavonoids in *C. asiatica*, is considered to be responsible for the health benefits associated with it (23, 25).

According to the data from the reviewed literature, it appears that the efficiency of extracting these bioactive constituents is influenced by the properties of the extraction solvents, extraction time, and extraction temperature (9, 20, 26). Flavonoids constitute a wide range of substances that play an important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules such as carbohydrates, proteins, lipids, and DNA. Roy (2018) and Das (2011) reported the presence of flavonoids in *Centella asiatica* leaves, suggesting that flavonoids can prevent oxidative reactions and improve the health status of humans and animals (11, 23).

Flavonoids exhibit antimicrobial, anti-inflammatory, and antitumor activities. They act as potent antioxidants that can protect the body against free radicals and reactive oxygen species (3, 12, 15).

The IC50 parameter is defined as the concentration (µg/ml) of a substance (extract) that causes a 50% loss of DPPH activity. In the case of the tested extracts, the inhibition percentage, which reflects their antioxidant activity, was calculated at 30 minutes, and the average of the three replicates is presented in Table 3. Figures 4, 5, and 6 also represent the results. Additionally, intermediate readings were taken at 5, 10, and 15 minutes, which are presented in Table 4. Based on the conducted determinations, the IC50 value was reached at an average concentration of 45.99 µg/ml for the ethanolic extract of *Centella asiatica*. Figures 3, 4, and 5 present IC50 for the ethanolic extract of *Centella asiatica.*

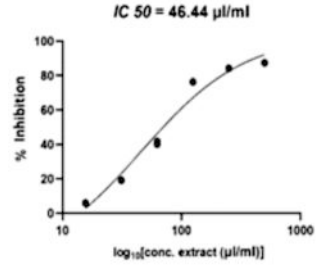

Fig. 3. IC 50 for *Centella asiatica* ethanol extract (replication 1)

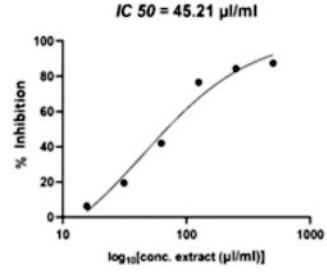

Fig. 4. IC 50 for *Centella asiatica* ethanol extract (replication 2)

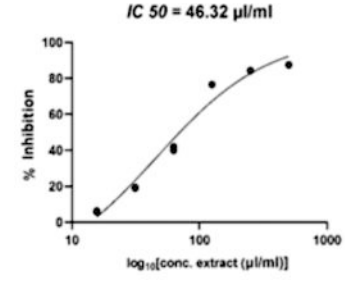

Fig. 5. IC 50 for *Centella asiatica* ethanol extract (replication 3)

Free radicals are among the factors that play a ma-

Table 4

Variation in the percentage of inhibition over time for the ethanol extract of *Centella asiatica*

Sample	µl/ml	5′ R1	5′ R2	5′ R3	Mean 5min	SD
Ethanolic extract CA	500	87.22	87.36	87.35	**87.31**	**0.08**
	250	83.95	84.11	84.07	**84.04**	**0.08**
	125	60.85	61.03	60.20	**60.69**	**0.44**
	62.5	37.28	37.98	38.17	**37.81**	**0.47**
	31.25	21.72	22.65	22.21	**22.19**	**0.46**
	15.61	11.06	11.47	11.56	**11.36**	**0.27**
		10′ R1	**10′ R2**	**10′ R3**	**Mean 10 min**	**SD**
	500	87.52	87.53	87.52	**87.52**	**0.00**
	250	84.44	84.42	84.40	**84.42**	**0.02**
	125	65.25	65.57	65.88	**65.56**	**0.32**
	62.5	38.73	38.69	38.52	**38.65**	**0.11**
	31.25	20.01	21.40	19.79	**20.40**	**0.87**
	15.61	9.13	9.55	8.94	**9.21**	**0.31**
		15′ R1	**15′ R2**	**15′ R3**	**Mean 15 min**	**SD**
	500	87.41	87.49	87.46	**87.45**	**0.04**
	250	84.43	84.42	84.49	**84.44**	**0.04**
	125	68.53	68.90	69.02	**68.82**	**0.26**
	62.5	38.93	41.00	41.16	**40.36**	**1.24**
	31.25	19.86	21.58	21.57	**21.00**	**0.99**
	15.61	6.56	11.50	11.12	**9.72**	**2.75**
		30′ R1	**30′ R2**	**30′ R3**	**Mean 30 min**	**SD**
	500	87.27	87.49	87.56	**87.44**	**0.15**
	250	84.06	84.30	84.39	**84.25**	**0.17**
	125	76.12	76.55	76.68	**76.45**	**0.29**
	62.5	40.75	41.95	40.00	**40.90**	**0.98**
	31.25	18.96	19.39	18.93	**19.09**	**0.25**
	15.61	5.88	6.25	5.71	**5.95**	**0.28**

jor role in the ageing process of the skin and can also be the cause of many dermatological diseases.

In the studies conducted by Sugunabai et al. (2015), it has been demonstrated that extracts of *Centella asiatica L.* effectively reduce the production of reactive oxygen species (ROS) in keratinocyte cells (27). Furthermore, several studies have described the remarkable protective effect of the plant against various diseases of the central nervous system (22, 31).

The data obtained from the statistical analysis for the ethanolic and methanolic extracts of *Centella asiatica*, comparing the inhibition percentage obtained at a concentration of 500 µg/ml with all the tested concentrations for the time intervals of 5, 10, 15, and 30 minutes, are presented in Fig. 6. According to the statistical analysis performed using the ANOVA test and the Bonferroni multiple comparison test with Graph Pad Prism 6.0 software (GraphPad Software, San Diego, CA, USA), we observed highly significant differences ($p < 0.0001$) in the inhibition percentage between the concentration of 500 µg/ml and the other concentrations used.

If we analyse the exposure time, we can observe highly significant differences ($p<0.0001$) at 15 and 30 minutes of exposure for the concentrations of 125, 62.5, 31.25, and 15.61 µg/ml. No significant differences were found between the exposure times for the concentrations of 500 and 250 µg/ml of ethanolic extract of *Centella asiatica.* Based on the obtained results, we can conclude that the antioxidant activity of ethanolic and methanolic extracts of *Centella asiatica* is dependent on and closely related to the concentration of bioactive compounds. Additionally, the extrac-

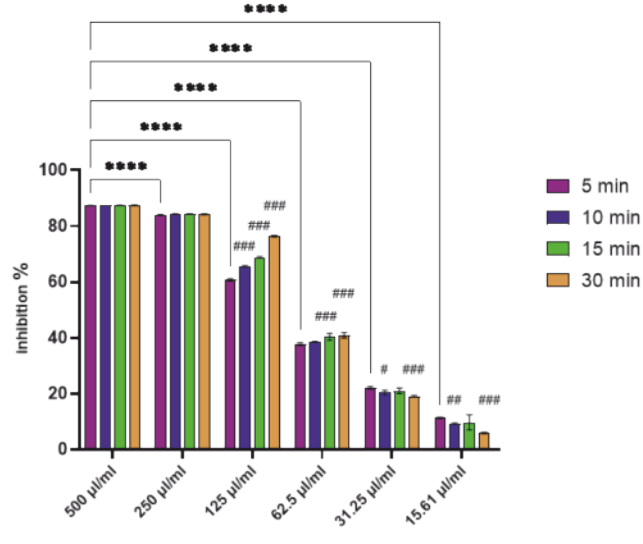

Fig. 6. Statistical analysis of the percentage of inhibition of *Centella asiatica* ethanolic extract at concentrations and exposure time. Comparison with 500 µl/ml: *** $p<0.0001$. Comparison between time exposure: # $p<0.05$, ## $p<0.01$, ### $p<0.001$

tion methods and types of solvents can affect the level of antioxidant properties of the *Centella asiatica* plant. The obtained results are consistent with those of other authors who argue that the free radical scavenging activity is significantly higher when ethanolic extracts are used. According to other studies, ethanolic extract has shown the highest antioxidant activities compared to extracts where the solvent was water, chloroform, or methanol. Recent studies have reported that the optimal extraction concentration for *C. asiatica* was achieved by dissolving the plant in 40% ethanol for 60 minutes at a solid/solvent ratio of 1:15 (9, 31).

CONCLUSIONS

Based on the conducted determinations, we can conclude that the ethanolic extract of *Centella asiatica* exhibits good antioxidant activity, which is correlated with the content of flavonoids and polyphenols. The IC50 value was reached at an average concentration of 45.99 µg/ml. As for the methanolic extract of *Centella asiatica*, no significant differences were observed in the inhibition percentage between the concentrations of 500 and 250 µg/ml.

However, these differences became highly significant ($p<0.0001$) between the concentration of 500 µg/ml and the other tested concentrations. Due to its rich chemical composition of potentially therapeutic compounds, *Centella asiatica* can be used in various therapeutic formulations, including for animals.

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REFERENCES

1. *Aftab A., Khan Z.D., Yousaf Z., Javad S., Shamsheer B., Zahoor M., Riaz N., Javed S., Yasin H., Ramzan, H*., (2017), Exploration of ethnopharmacological potential of antimicrobial, antioxidant, anthelmintic and phytochemical analysis of medicinally important plant *Centella asiatica* (L.) Urban in Mart. and Eichl. American Journal of Plant Sciences, 8(2):201-211
2. *Al-Matani S.K., Al-Wahaibi R.N.S., Hossain M.A.*, (2015)., Total flavonoids content and antimicrobial activity of crude extract from leaves of *Ficus sycomorus* native to Sultanate of Oman. Karbala International Journal of Modern Science, 1(3):166-171
3. *Alothman M., Bhat R., Karim A.A*., (2009), Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry, 115:785-788
4. *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. Biomed Pharmacother, 105:1344-1352
5. *Babykutty S., Padikkala J., Sathiadevan P.P., Vijayakurup V., Azis T.K., Srinivas P., Gopala S.,* (2008), Apoptosis induction of *Centella asciatica* on human breast cancer cells. Afr J Tradit Complement Altern Med, 6(1):9-16
6. *Bhavna D., Khatri J.,* (2011), Review of *Centella asiatica*: The Elixir of Life. Int J Res Ayurveda Pharmacy, 2:431-438
7. *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. Phytother Res, 35:6624-6654
8. *Cheng C.L., Guo J.S., Luk J., Koo M.W.L.*, (2004), The healing effects of *Centella* extract and asiaticoside on acetic acid induced gastric ulcers in rats. Life Sci, 74:2237-2249
9. *Chew K.K., Ng S.Y., Thoo Y.Y., Khoo M.Z., Wan Aida W.M., Ho C.W,* (2011), Effect of ethanol concentration, extraction time, extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Centella asiatica* extratcs. International Food Research Journal, 18:571-578
10. *Chippada S.C., Vangalapati M.,* (2011), Antioxidant, an antiinflammatory and anti-arthritic activity of *Centella asiatica* extracts. Journal of Chemical, Biological and Physical Sciences, 1(2):260
11. *Das A.J.,* (2011), Review on nutritional, medicinal and pharmacological properties of *Centella asiatica* (Indian pennywort). Journal of Biologically Active Products from Nature, 4:216-228
12. *Dharmendra S., Abhislick G.,* (2013), Phytochemistry of medicinal plants. Journal of Pharmacognosy and Phytochemistry, 1(6):168-182
13. *Ferdous N., Rahman M., Alamgir A.N.M*., (2017), Investigation on phytochemical, cytotoxic and antimicrobial properties of ethanolic extracts of *Centella asiatica* (L.) Urban. J Med Plants Stud, 5:187-188
14. *George M., Joseph L, Ramaswamy,* (2009), Anti-allergic, anti-pruritic, and anti-inflammatory activities of *Centella asiatica* extracts. Afr J Trad CAM, 6(4):554-559
15. *Gupta S., Gown B.S., Lakshmi A.J., Prakash J.,* (2013), Retention of nutrients in green leafy vegetables on dehydration. The Journal of Food Science

and Technology, 50(5):918-925
16. *Hou Q., Li M., Lu Y.H., Liu D.H., Li C.C.,* (2016), Burn wound healing properties of asiaticoside and madecassoside, Exp Ther Med, 12(3):1269-1274
17. *Jayaprakash S.B., Nagarajan N.,* (2016), Studies on the bioactive compounds and antimicrobial activities of medicinal plant *Centella asiatica* (Linn), J Med Plants Stud, 4:181-185
18. *Liu M., Dai Y., Li Y., Luo Y., Huang F., Gong Z., Meng* Q., (2008), Madecassoside isolated from *Centella asiatica* herbs facilitates burn wound healing in mice. Planta Med, 74:809-815
19. *Mamtha B., Kavitha K., Srinivasan K.K., Shivananda P.G.,* (2004), An *in vitro* study of the effect of *Centella asiatica* [Indian pennywort] on enteric pathogens. Indian J of Pharm, 36:41
20. *Ondeko D.A., Juma B.F., Baraza L.D., Nyongesa P.K.*, (2020), LCESI/MS and GC-MS methanol extract analysis, phytochemical and antimicrobial activity studies of *Centella asiatica*. Asian J Chem Sci, 8(3):32-51
21. *Prakash V., Jaiswal N., Srivastava M., (*2017), A review on medicinal properties of *Centella asiatica*. Asian Journal of Pharmaceutical and Clinical Research, 10(10):69-74
22. *Pittella F., Dutra R.C., Junior D.D., Lopes M.T.P., Barbosa N.R.,* (2009), Antioxidant and cytotoxic activities of *Centella asiatica* (L). Int J Mol Sci, 10:3713-3721
23. *Roy A.R.,* (2018), Qualitative and quantitative phytochemical analysis of *Centella asiatica*. Natural Products Chemistry and Research, 6(4):1000323
24. *Seevaratnam V., Banumathi P., Premalatha M.R., Sundaram S.P., Arumugam, T.,* (2012), Functional properties of *Centella asiatica* (L.): a review. International Journal of Pharmacy and Pharmaceutical Sciences, 4(5):8-14
25. *Singh S., Gautam A., Sharma A., Batra A.,* (2010), *Centella asiatica* (L.): a plant with immense medicinal potential but threatened. Int J Pharmaceut Sci Rev Res, 4:9-17
26. *Subban R., Veerakumar A., Manimaran R., Hashim K.M., Balachandran I.,* (2008), Two new flavonoids from *Centella asiatica* (Linn.). J Nat Med, 62(3): 369-373
27. *Sugunabai J., Jeyaraj M., Karpagam T.,* (2015), Analysis of functional compounds and antioxidant activity of *Centella asiatica*. World J Pharm Pharm Sci, 4(8):1982-1993
28. *Sultan R.A., Mahmood S.B.Z., Azhar I., Ahmed S.W., Mahmood Z.A.,* (2014), Biological activities assessment of *Centella asiatica* (Linn.), J. Herbs-spices Med Plants, 20:319-327
29. *Sun B., Wu L., Wu Y.,* (2020), Therapeutic potential of *Centella asiatica* and its triterpenes: a review. Front Pharmacol, 11:568032
30. *Tamas-Krumpe O.M., Bobiș O., Mărgăoan R., Doștețan Abălaru C., Buta A., Ognean L.,* (2010), The analysis of the biologically active compounds content and the antioxidant potential of some Romanian polyfloral honey samples. Filodiritto Editore – Proceedings, 1010-1019
31. *Tan P.W., Tan C.P., Ho C.W*., (2011), Antioxidant properties: Effects of solid-to-solvent ratio on antioxidant compounds and capacities of pegaga (*Centella asiatica*). International Food Research Journal, 18:557-562
32. ***, (2000), Romanian Pharmacopoeia, 10th Edition [in Romanian], (Ed.) Medicala, Bucharest, Romania.