Journal of Academia and Industrial Research (JAIR)
Volume 10, Issue 3, Pages 26-29

ISSN: 2278-5213

Research Article

Proximate analysis and phytochemical Extraction from *Centella asiatica* and its analysis by HPLC method

G. Prem Kumar* and Prabhavathi Prem Kumar
[1, 2]Crescent Biologicals Pvt. Ltd., Gandhipet, Tirupattur, Tamil Nadu, India
drpremkumar@crescentbiologicals.com*; +91 93440 37811
Received: February 04 2022/Accepted: April 30 2022/Published: 07 May 2022

Abstract

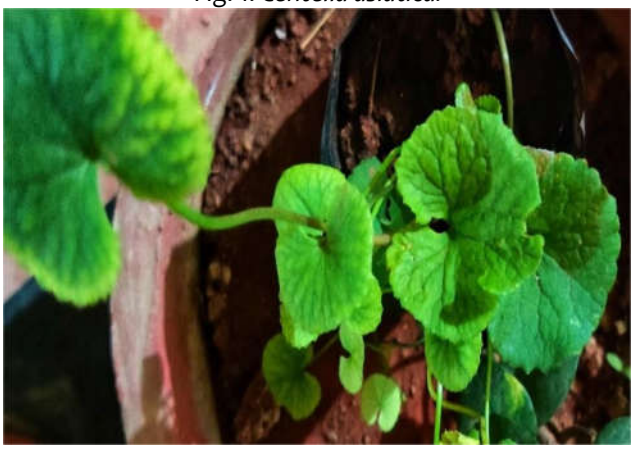
The conventional method of extracting triterpenes by the use of organic solvents from *Centella asiatica* is of greater interest by many investigators due to its use in health sectors to prevent and treat various diseases. In this study, the conventional extraction to enhance the bioavailability of phytochemicals at different concentrations was performed using ethanol. On extraction, yield of asiaticoside from *Centella asiatica* was examined for temperature and time consideration effect of extraction time (2 h). The sample was subjected to proximate analysis like fat analysis by Soxhlet apparatus, protein analysis by kjeldhal titration, ash analysis by muffle furnace and drying (loss on drying) by hot air oven respectively. The estimation of triterpenes, asiaticosides was determined using High performance liquid chromatography (HPLC) and the assay percentage was determined as 6.114%. The concentration of ethanol and temperature showed significant impact on the yield of finished product. With 2 h of extraction, the proper content of polyphenol was observed in 80% ethanol. Raw material attributed to high protein (13.81%), fat content of 3.085% and the solubility of finished product in water was about 98.02%.

Keywords: *Centella asiatica,* triterpenes , asiaticoside, polyphenol, protein content, solubility.

Introduction

Centella asiatica is a tropical medicinal herb of Umbellifera family and natively found in south-east Asian countries such as China, Japan, India, Srilanka, Indonesia and Malaysia also in South Africa. *Centella asiatica* commonly known as "Gotukola", Indian pennywort, wild violet in English is a Tropical plant (Fig. 1). Due to its medicinal value and its importance, it is cultivated all over globally. The leaves are edible, yellowish green colour, thin, alternate with long petioles and quite characteristics reniform orbicular or oblong–elliptic shapes with seven veins. Through its green to red stolones the plant grows horizontally which combine to each other and roots in underground. *Centella asiatica* has very unique property and many pharmacological uses such as wound healing as it contains bioactive components such as madecassol, madecassic acid, asiatic acid and asiaticosides. It is mild sedative and has anti-anxiety properties which increase memory function by increasing the flow of blood to the brain (Nnakaca *et al.,* 2020). It also acts as cardioprotective, radioprotective, anti-depressant and immunomodulators (Singh, 2010; Agme and sagar, 2016).

Fig. 1. *Centella asiatica.*



Centella asiatica is basically used in various disease treatments such as lupus, ulcers, eczema, psoriasis, diarrhea and skin condition like leprosy, fever, amenorrhea and diseases of female genitourinary tract (Gray *et al.,* 2017). This plant mainly deals with the property of wound healing and memory enhancement effects according to World Health Organization (Orhan, 2012; Gupta and Bhatt, 2018).

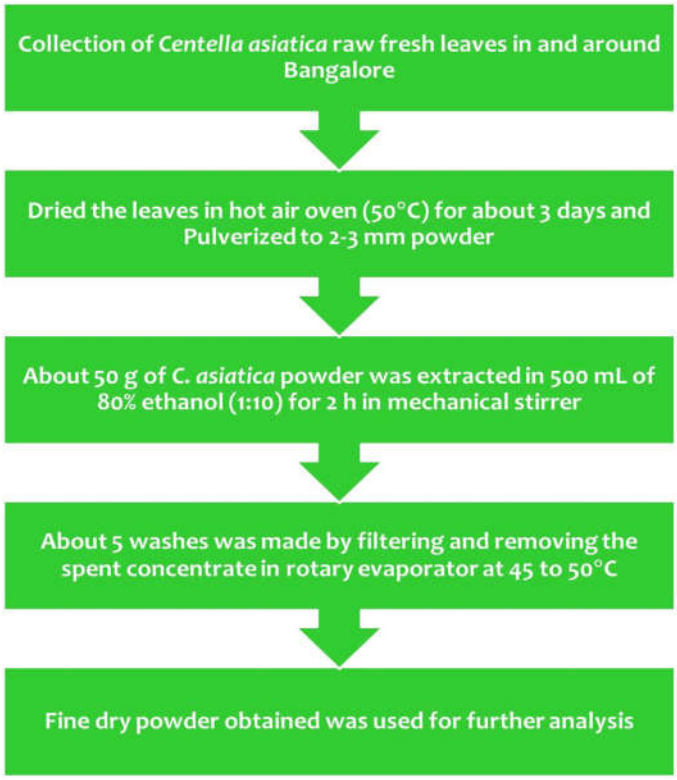
***Corresponding author**

Centella asiatica has bioactive compounds like asiatic acid, asiaticoside which shows better effect against bacteria and fungi. Asiaticosides are triterpenes which has therapeutic effect. Asiatic acid is an aglycone form of Asiaticoside and easily available formed by hydrolyzing the sugar moiety of asiaticoside structure in acid condition and some data suggest that therapeutic effect of Asiaticoside comes from Asiatic acid. To increase the bioavailability of the bioactive components such as asiaticoside, the conventional method of extraction by using ethanol and methanol was performed by HPLC under the absorbance of 210 nm and the average recovery (Jain and Agarwal, 2008; Roy *et al.*, 2013). Hence this study focuses on the proximate analysis and phytochemical extraction from *Centella asiatica* and its analysis by HPLC method.

Materials and Methods

Sample collection: *Centella asiatica* leaves were collected in and around Bangalore, Karnataka, India. The detailed extraction procedure is given in Fig. 2.

Fig. 2. Extraction of phytochemicals from *Centella asiatica.*



Fat analysis: For the analysis of fat content, *C. asiatica* 1 g of the sample was added to 100 mL of hexane in round bottom flask and placed on the Soxhlet extractor at 40°C for 2 h. After 2 h, the contents were filtered and the filtrate was taken in an evaporating dish and kept on the heating mantle for complete evaporation of the filtrate. The weight of the empty evaporating dish was noted before and taken as W1; the dried filtrate in the dish is taken as W2. Fat %=W2 - W1/weight of the sample taken x 100.

Ash analysis: About 2 g of the sample is weighed in the dried crucible, before weighing the weight of empty crucible is noted down as W1. Then the crucible is placed on the hot plate for 1 h and then placed in the muffle furnace at 630°C for 3 h till the sample is completely charred. The weight is noted down as W3. Ash % = W3-W2/W2-W1 x100

Loss on drying (LOD): First the weight of the empty Petri plate is noted down as W1. Then 2 g of the sample is weighed accurately and taken as W2. Then the petri plate was placed in the hot air oven for 2 h. Then the petri plate was taken out and allowed to cool down in the desiccator for 5 min and it is weighed using the weighing balance. Note down as W3. LOD % = W3-W2W2-W1 x 100.

Protein analysis: The sample of 200 mg was weighed and added to all digestion tubes. Then 3.5 g of catalyst mixture (potassium sulfate + copper sulfate) and 10 mL of concentrated H_2SO_4 was added to the digestion tubes and kept for digestion in Kjeldhal digestor unit. The sample was heated below the boiling point until frothing ceases and increased the heat until the acid boils vigorously and the sample was digested for 1 h. After complete digestion, the solution turns light green to blue colour, about 10-15 mL of de-mineralized water was added to cool the digestion tubes. Then 40 mL of 40% NaOH and 20 mL of de-mineralized water was added to the digestion tubes and placed in the distillation unit. On the other hand, 20 mL of 2% boric acid was added in the conical flask and the tip tube was placed. Switched on the distillation unit and the steam were provided until all the ammonia has passed to the conical flask containing 2% boric acid. Then, 2-3 drops of methyl red indicator was added to the distillate and titrated against 0.1 N HCl till the pale pink colour appears which marks the end point. Similarly blank is carried out by omitting the sample. Protein (%) = (A-B) X N X 1.401/W X 6.25. Where A is the Volume of 0.1 N HCl used for sample titration, B is the volume of 0.1 N HCl used for blank titration and N is the normality of HCl and W is the weight of the sample. The value 1.041 is the factor containing the molecular mass of nitrogen. Protein–nitrogen conversion factor is 6.25.

Analysis of asiaticosides by HPLC: Asiaticoside was assayed by HPLC (Shimadzu) using a gradient of ACN (acetonitrile) and 0.3% phosphoric acid (flow rate of 1.8 mL/min) using UV detector at 210 nm. Accurately 50 mg of working standard was added into 25 mL of volumetric flask and dissolved with HPLC grade methanol and diluted up to the mark with MeOH.

*Corresponding author

The standard and the sample were separately injected with equal volume in to chromatograph (4.6 × 25 cm; Eclipse plus c18 250 x 4.60 mm, 5 microns; Run time of 40 min) and measured the peak responses for the compound. The assay percentage of *Centella asiatica* was calculated as asiaticoside assay%=Sample area × standard weight × sample dilution × purity of standard/Standard area × standard dilution × sample weight.

Solubility test: About 1 gram of sample was dissolved in 100 mL of water and sonicated for complete dissolution and the sample was filtered by Whatman filter paper. Percentage of insoluble solubility = Residue weight × 100/ Weight of sample; percentage of solubility=100–insolubilities.

Results and Discussion

As per the trials and yield percentage, the concentration of the ethanol plays important role in obtaining the yield. The yield obtained from 80% of ethanol was more compared to the 90% of ethanol. The more concentration of ethanol leads to improper yield, so as per the results 80% concentrated ethanol was favorable for increasing the bioavailability of the asiaticoside. Trial 4 and 5 accurately represents the increased yield of the final product (Table 1).

Proximate analysis of Centella asiatica: The results of the proximate analysis of *C. asiatica* are presented in Table 2. The lipid content is least and ash content is highest and these are obtained in 20% of finished product of trial 4 extraction. The input ratio of the lipid content of raw material is less than that of finished product that i.e. 1. 46 and 3.085%. The protein content is high (13.81%) when compared to the finished product (5.92%). The ash analysis revealed that the raw material was having more ash content (15.44%) compared with that of 10.74% in the finished product. The loss on drying results was 3.97% for raw material and 5.15% for finished product. The proximate analysis revealed that *C. asiatica* leaves had an appreciable amount of Ash and protein. The high ash content indicates good mineral sources for human nutrition. The substantial amount of fiber shows that the leaves of *C. asiatica* can help in keeping the digestive system healthy by removing toxicants from the body and prevents the absorption of excess cholesterol. So, the overall result revealed that *C. asiatica* leaves are rich sources of proteins and lipids.

Solubility check: The finished product was soluble in only few organic solvents such as ethanol and methanol and insoluble in other solvents. The amount of product soluble in water was about 98.02% and hence it is hydroscopic substance in nature.

Table 1. Extraction trials and percentage of yield obtained.

Trial No.	Sample input	Ethanol conc.	Final yield	Yield %
Trial-1	50 g	80%	5.268 g	10.53
Trial-2	25 g	80%	3.7628 g	5.05
Trial-3	50g	90%	-	-
Trial-4	50 g	80%	10.1178 g	20.23
Trial-5	50 g	80 %	11.2140g	22.42

Table 2. Proximate analysis of *Centella asiatica*.

Contents	Raw material	Finished product
Lipid	1.46%	3.085%
Protein	13.81%	5.92%
Ash	15.446%	10.7424%
Loss on dryness	3.979%	5.15%
Moisture	12.08%	-

Estimation of asiaticoside: HPLC analysis proceeded with standard calibration curves that are constructed from the chromatograms for the separation of standard asiaticoside. The Asiaticoside assay% was determined as 6.114% (Fig. 3 & 4). The HPLC analysis of *C. asiatica* showed asiaticoside peak at a retention time of 10.165 min which was compared with the standard 10.200 min. Asiaticoside is an active bioactive molecule plays many pharmacological activities such as neuroprotective, anticancer, angiogenic, antipyretic and cognition enhancing activities.

Fig. 3. HPLC chromatogram of standard asiaticoside.

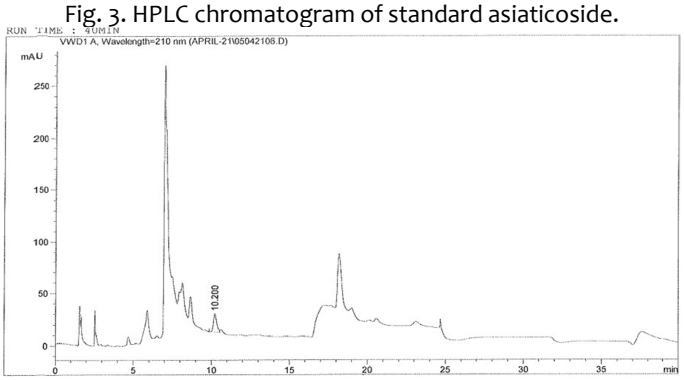
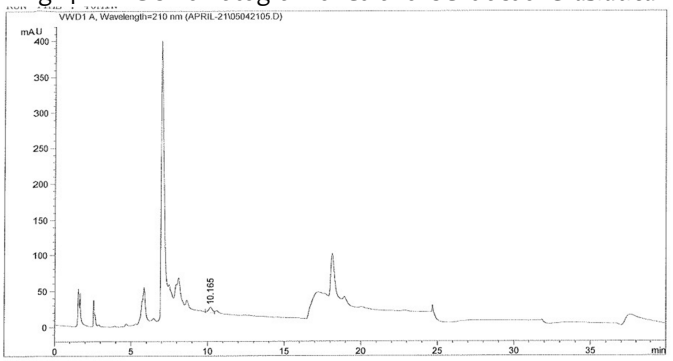


Fig. 4. HPLC chromatogram of ethanolic extract of *C. asiatica.*



*Corresponding author

Conclusion

The conventional extraction to enhance the bioavailability of phytochemicals from *Centella asiatica* at different concentrations was performed using ethanol. On extraction, yield of asiaticoside from *Centella asiatica* was examined for temperature and time consideration effect of extraction time (2 h). The sample was subjected to proximate analysis like fat analysis by Soxhlet apparatus, protein analysis by kjeldhal titration, ash analysis by muffle furnace and drying (loss on drying) by hot air oven respectively. The estimation of triterpenes, asiaticosides was determined using High performance liquid chromatography (HPLC) and the assay percentage was determined as 6.114%. The concentration of ethanol and temperature showed significant impact on the yield of finished product. With 2 h of extraction, the proper content of polyphenol was observed in 80% ethanol. Raw material attributed to high protein (13.81%), fat content of 3.085% and the solubility of finished product in water was about 98.02%. Asiaticoside is an active bioactive molecule plays many pharmacological activities such as neuroprotective, anticancer, angiogenic, antipyretic and cognition enhancing activities.

References

1. Agme, V. and Sagar, R. 2016. Analysis of bioactive compounds in leaves extract of *Centella asiatica* by using HRLC-MS and IR techniques. *J. Chem. Pharmaceut. Res.* 8(8): 122-125.
2. Bylka, W., Znajdek-Awiżen, P., Sroka, E.S., Dańczak-Pazdrowska, A. and Malgorzata. 2014. *Centella asiatica* in dermatology: an overview. *Pytotherp. Res.* 28(8): 1117-1124.
3. Gray, N.E. and Magana, A.A. 2017. *Centella asiatica:* Phytochemistry and mechanism of neuroprotection and cognitive enhancement. *J. Chem. Pharmacol. Res.* 18: 17-29.
4. Gupta, S. and Bhatt, P. 2018. Determination and quantification of asiaticoside in endophytic fungus from *Centella asiatica* (L.) urban. *World J. Microbiol. Biotechnol.* 34: 111-121.
5. Jain, P.K. and Agarwal, R.K. 2008. High performance liquid chromatographic analysis of asiaticoside in *Centella asiatica* (L.) urban. *Chiang Mai J. Sci.* 1826: 102-110.
6. Nnokacu, K., Fu, I. and Peteroj, F. 2020. Nutrient and phytochemical composition of *Centella asiatica* leaves. *Med. Arom. Pl.* 346: 26-29.
7. Orhan, I.E. 2012. Therapeutic approaches to neuroprotective activity by complementary and alternative medicines. *Hindawi J.* 94: 62-79.
8. Roy, D.C., Barman, S.K. and Shaik, M. 2013. Current updates on *Centella asiatica:* Phytochemistry, pharmacology and traditional uses. *Med. Pl. Res. J.* 3: 4-9.
9. Singh, S., Gautam, A. and Shrama, A. 2010. *Centella asiatica* (L): A plant with immense medicinal potential but threatened. *Int J. Pharmaceut. Sci. Rev. Res.* 4(9): 17-25.
10. Tareq, M. and Khan, M. 2012. *Centella asiatica* (L) urban; from traditional medicine to modern medicine with neuroprotective potential. *Hindawi J.* 10: 115-126.

Cite this Article as:

Prem Kumar, G. and Prabhavathi Prem Kumar. 2022. Proximate analysis and phytochemical extraction from *Centella asiatica* and its analysis by HPLC method. *J. Acad. Indus. Res.* 10(3): 26-29.

***Corresponding author**