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Research Article

Assessment of Therapeutic Potential and Phytochemical Profiling in Different Solvent Extracts of *Centella asiatica*

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Abstract

Centella asiatica (L.) Urban, commonly known as Indian pennywort, a small perennial herb from the Apiaceae family has been used in traditional medicine for thousands of years. This study aimed to evaluate the effect of different extraction solvents on the therapeutic and phytochemical profiles of various parts of *Centella asiatica*. Among the various plant parts assessed, the leaves exhibited the best antioxidant and in vitro antidiabetic activity and the highest amount of phytoconstituents compared to petiole and root. Methanol was identified as the most effective solvent, yielding optimal IC50 values for DPPH (30.65 µg/ml) and ABTS (45.23 µg/ml). Additionally, the methanolic extract of leaves exhibited highest content of phenolics (65.49 mg GAE/g DW) and flavonoids (12.56 mg QE/g DW). The methanolic leaf extract also demonstrated a promising inhibition potential against α-amylase and α-glucosidase enzymes and possessed elevated levels of alkaloids, glycosides, tannins and terpenoids. This study demonstrated significant variations in the therapeutic potential of *Centella asiatica*, attributed to differences in the nature of the extraction solvents.

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Introduction

Plants serve as fundamental source of knowledge for medicine, playing a crucial role in both traditional and modern medical systems globally. Natural substances derived from plants have been used for detecting and managing various diseases since ages. Due to their safety, accessibility, availability, tolerance and low toxicity, plants are an excellent source for developing therapeutic drugs. The diverse phytochemicals in herbal medicine attract researchers aiming to create novel treatments for numerous ailments (Harwansh et al., 2023; Bansal et al., 2024). Additionally, there is growing interest in developed countries in using natural remedies for healthier and more balanced lifestyles.

Centella asiatica (L.) Urban, commonly known as Gotu kola, mandukparni, Indian pennywort, jalbrahmi and pegaga nyonya, is a small perennial herb from the Apiaceae family. Historic texts such as the "miracle elixirs of life" and the "Sushruta Samhita" highlight its medicinal use in ancient Chinese and Indian medicine. It is also traditionally used in Sri Lanka and other Indonesian islands (Gohil et al., 2010; Diniz et al., 2023). *Centella asiatica* is a creeping plant that spreads through stolons, featuring shovel-shaped leaves with scalloped edges, borne on elongated petioles grouped at stem nodes. It produces small green or pinkish-white flowers in dense umbels, and its seeds are pumpkin-shaped nutlets, 3-5 mm long. Due to its extensive medicinal properties, it is used both as a modern and traditional botanical remedy and as a salad vegetable or in juices (Poovizhi et al., 2022).

It is widely consumed as a health drink and as a vegetable in various Asian cuisines. It has been a cornerstone in Ayurvedic and Chinese traditional medicine for centuries, treating dermatological conditions like bacterial infections, psoriasis, scleroderma, ulcers, leprosy and skin inflammation from wounds and burns. Additionally, it has demonstrated neuroprotective, memory-enhancing, antidepressant, and anxiolytic effects in neurological applications (Tassanawat et al., 2013; Hengjumrut et al., 2018; Tan et al., 2021) . Due to its effectiveness, accessibility, cost efficiency, and low toxicity,

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Centella asiatica extracts have been clinically investigated in various fields.

The herb contains compounds such as phenolic acids, triterpene steroids, volatile oils, flavonoids, tannins, phytosterols, vitamins, essential oils, amino acids, and sugars. Saponins and their aglycones are the most abundant pentacyclic triterpenoids, with asiaticoside and madecassoside comprising about 8% of the herb's dry mass. However, the quantity of triterpene constituents varies based on geographical origin, genetics, environmental factors and growth conditions (Hashim et al., 2011; Bandopadhyay et al., 2023).

Solvent extraction is widely regarded as the most effective technique for isolating bioactive compounds from plants. The yield and therapeutic properties of these extracts are significantly influenced by the choice of solvent (Sultana et al., 2009), due to the varying chemical characteristics and polarities of different phytochemicals, which affect their solubility (Truong et al., 2019). Therefore, selecting the appropriate extraction solvent for specific plant materials can be challenging. The present study aims to investigate the effect of solvent extraction on the therapeutic potential of various parts of *Centella asiatica* , specifically the leaves, petiole and root.

Materials and Methods

Plant Material

The fresh plant materials of *Centella asiatica* (figure 1) were collected rinsed thoroughly and later blotted dry to remove any excess moisture. The various parts of *Centella asiatica* , namely leaves, petiole and root were taken shade dried and uniformly powdered for extraction.

Extraction

The powdered leaves, petiole and root of *Centella asiatica* were separately extracted with methanol, distilled water and acetone using soxhlet apparatus for 24 hrs. The solvent was distilled at lower temperature under reduced pressure and concentrated on water bath to get the crude extract for future analysis.

DPPH based free radical scavenging activity

The DPPH free radical scavenging activity of the extracts was determined following the method (Blois, 1958). The reaction mixture contained 1.8 ml of 0.1 mM DPPH and 0.2 ml of different extracts. The absorbance of the reaction mixture was measured at 517 nm after an incubation of 30 min. A reaction mixture without test sample was considered as control.

ABTS+ radical cation(s) decolorization assay

The spectrophotometric analysis of ABTS+ radical cation(s) scavenging activity was measured according to Re et al. (1999) method. 1 ml of ABTS+ solution was reacted with 0.5 ml of extract. After 30 min of incubation at room temperature the absorbance was recorded at 734 nm. Solvent blanks were run in each assay.

Reducing antioxidant power (FRAP) Assay

The reducing antioxidant power of the extracts was estimated by the standard method (Oyaizu, 1986). The extracts were mixed with 0.2 M of phosphate buffer (2.5 ml, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The reaction mixture was kept safely for incubation at 50^{o} C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid was added to reaction mixture and centrifuged at 3000 rpm. The supernatant (1 ml) was taken and diluted with distilled water (1 ml) and 0.2 ml of $FeCl_3$ (0.1%) added to it. The increase in OD value was recorded at 700 nm against a blank.

In vitro α-Amylase Inhibitory Activity

The α-amylase inhibition potential of the extract was measured using a standard spectrophotometric method (Paul and Banerjee, 2013). To conduct the assay, 0.5 ml of reconstituted aqueous extract was mixed with 0.5 ml of α-amylase solution and incubated at 37°C for 5 minutes. After incubation, 0.5 ml of 1% starch solution was added, and the mixture was incubated for an additional 10 minutes. To stop the reaction, 1 ml of DNSA reagent was added and the mixture was heated in a hot water bath for 10 minutes until the color changed to orange-red. The reaction mixture was then cooled and diluted to 5 ml with distilled water. The absorbance was measured at 540 nm. The α-amylase inhibitory activity was determined by the concentration of inhibitor required to inhibit 50% of the enzyme activity.

In vitro α-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity of the extract was assessed using a method adapted from Jung et al. (2006) with minor modifications. Different concentrations of the extract were prepared using 0.2 mM phosphate buffer (pH 6.8). To each sample, 0.1 ml of enzyme solution was added and incubated at 37°C. Subsequently, 0.25 ml of 3 mM pNPG was added, and the reaction was terminated by adding 4 ml of 0.1 M Na_2CO_3. The release of pNPG was

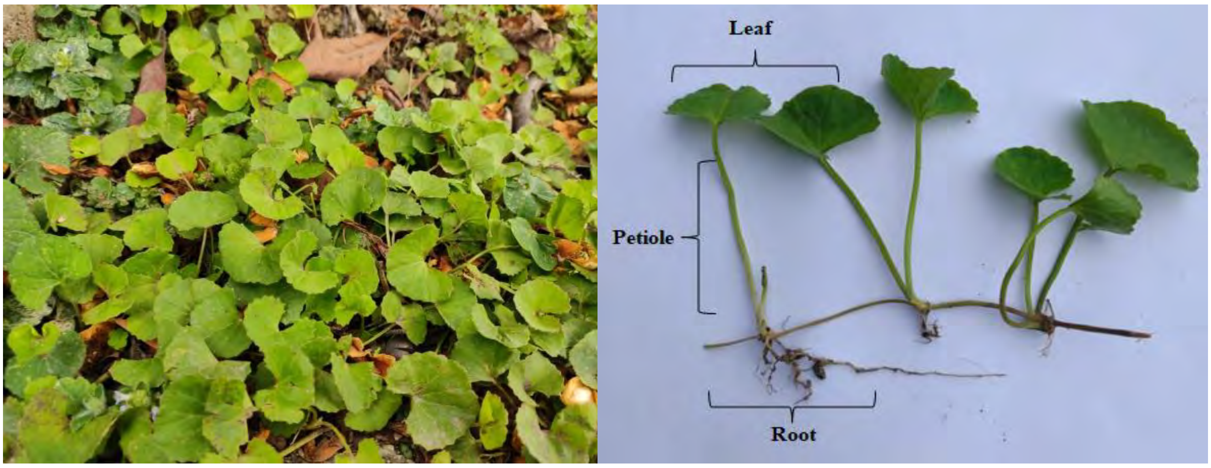


Fig 1. Different parts of *Centella asiatica* used for the present study

measured at 405 nm. A control sample containing all reagents without the extract was also prepared. The α-glucosidase inhibitory activity was calculated using the following equation:

Inhibitory ratio (%) = [1 - (As - Ab) / Ac] × 100

where As, Ab and Ac represent the absorbance values of the sample, blank and control reaction mixtures, respectively.

Total phenolic content

Total phenolic contents (TPC) of the extracts were measured according to the standard protocol (Chandler and Dodds, 1993). 1 ml of extract was reacted with a mixture containing 1 ml of 95% ethanol solution, 5 ml of distilled water and 500 µl of folin-ciocalteau reagent (50%). After incubation period of 5 min, 1 ml of 5% Na_2CO_3 was added. It was mixed thoroughly on a vortex shaker and further kept for 1h of incubation at room temperature. Finally, the absorbance of coloured reaction mixture was recorded at 765 nm against the reagent blank. The total phenolic content was measured as mg of Gallic acid equivalent per gram dry weight (DW).

Total flavonoid content

The total flavonoid content (TFC) was measured by performing a standard spectrophotometric method with some modifications (Zhishen et al., 1999). 1 ml of extract was diluted with 4 ml distilled water in a volumetric flask. Initially, 300 µl $NaNO_2$ solution (5%) was added to each volumetric flask and after 5 min, 0.3 ml $AlCl_3$ (10%) was also added. After 6 min of incubation, 2 ml NaOH (1 M) was added to the mixture. Absorbance of the reaction mixture was measured at 510 nm after adding 2.4 ml of distilled water. The flavonoid content in different extracts was estimated as quercetin equivalent (QE) per gm dry weight (DW).

Phytochemical analysis

Phytochemical screening was performed to detect the primary groups of secondary metabolites present in the leaves, petiole and root of *Centella asiatica* by using the standard procedures. The phytochemicals like alkaloids, glycosides, saponins, tannin and terpeniods were analysed following the standard methods with some modifications (Kumar et al., 2009; Wadood et al., 2013; Yusuf et al., 2014; Banu et al., 2015).

Statistical analysis

The statistical tools such as MS Excel 2007 (Microsoft, Redmond, WA, USA), DSAASTAT software (version 1.002; DSAASTAT, Peruglia, Italy), Smith's Statistical Package version 2.5 (prepared by Gary Smith, CA, USA) and Multivariate Statistical Package (MVSP 3.1) were used for statistical analysis of data.

Results and Discussion

Assessing the antioxidant potential of plant extracts is crucial for determining their nutritional value (Ghoshal and Mandal, 2013). In this study, the antioxidant properties of extracts from the leaves, petiole and root of *Centella asiatica* were evaluated. The results showed that leaf extracts exhibited the highest antioxidant activity. Among the solvents tested, methanol yielded the best results, making it the preferred solvent for extracting bioactive compounds. The methanolic leaf extract exhibited IC50 values of 30.65 µg/ml and 45.23 µg/ml for DPPH and $ABTS^+$ scavenging activity, respectively (Figure 2). These findings suggest that leaf extract has a strong ability to neutralize free radicals, making it a potent agent against oxidative stress-related disorders. In this study, reducing power was

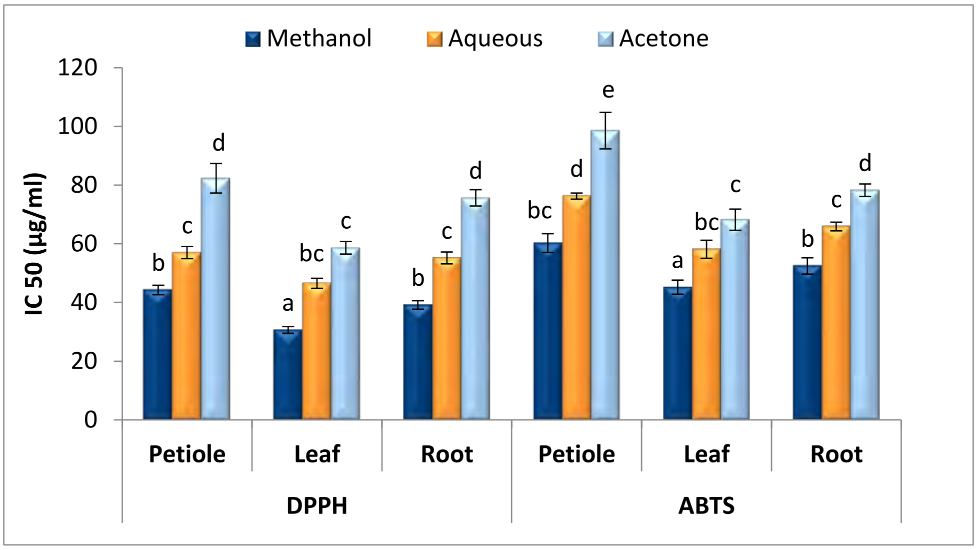


Fig 2. Free radical scavenging activity of different parts of *Centella asiatica* obtained by various extraction solvents. Results are represented as mean±SEM, n=3. Values with different letters (a, b and c) represent significant ($P< 0.05$) differences of means.

measured in ascorbic acid equivalents (AAE); higher AAE values indicate stronger reducing power. The AAE values ranged from 94.76 to 208.16 AAEµg/g DW, with methanolic leaf extracts showing the highest values, followed by petiole and root (Table 1). Methanolic extracts consistently exhibited better activity than aqueous and acetone extracts across all plant parts. Previous literature indicates that solvent choice significantly affects extraction yield and bioactive compound content, impacting biological activity (Sultana et al., 2009; Ngo et al., 2017). Our findings align with this trend, showing significant variation in bioactive phytochemical content and antioxidant activity across different solvent extracts (Figure 2; Tables 2 & 3). Key enzymes in the digestive system, α-amylase and glucosidase, are crucial for the breakdown of starch into glucose, which is released into the bloodstream. Inhibiting these enzymes can reduce starch breakdown, potentially lowering post-prandial hyperglycemia levels (Tarling et al., 2008). This study evaluated the *in vitro* antidiabetic activity of various parts of *Centella asiatica* by determining their ability to inhibit α-glucosidase and α-amylase, expressed as IC50 values. The results demonstrated that *Centella asiatica* possess significant antidiabetic properties and the inhibitory potential much stronger against α-glucosidase compared to α-amylase. However, the leaf extract exhibited best IC50 values of 96.67 µg/ml and 108.96 µg/ml against α-glucosidase and α-amylase, respectively (Figure 3). Phenolic and flavonoid compounds, known for inhibiting α-glucosidase and regulating hyperglycemia (Lin et al. 2016), were found in higher concentrations in extracts with greater phenolics play a vital role in managing diabetic disorders by inhibiting these key digestive enzymes.

Table 1. Reducing power activity of different parts of *Centella asiatica* obtained by various extraction solvents. Values represent mean ± SD (n = 3). Superscript letters within the same row indicate significant ($P< 0.05$) differences of means within the plant parts; Subscript letters within the same column indicate significant ($P< 0.05$) differences of means within the extraction solvent

	FRAP AAE µg/g DW		
	Petiole	**Leaf**	**Root**
Methanol	173.18±4.15$^{b}_{a}$	208.16±6.72$^{a}_{a}$	165.85±3.58$^{b}_{a}$
Aqueous	141.32±3.96$^{b}_{b}$	189.42±4.22$^{a}_{b}$	120.67±6.33$^{c}_{b}$
Acetone	94.76±3.62$^{b}_{c}$	119.53±5.02$^{a}_{c}$	94.89±3.89$^{b}_{c}$

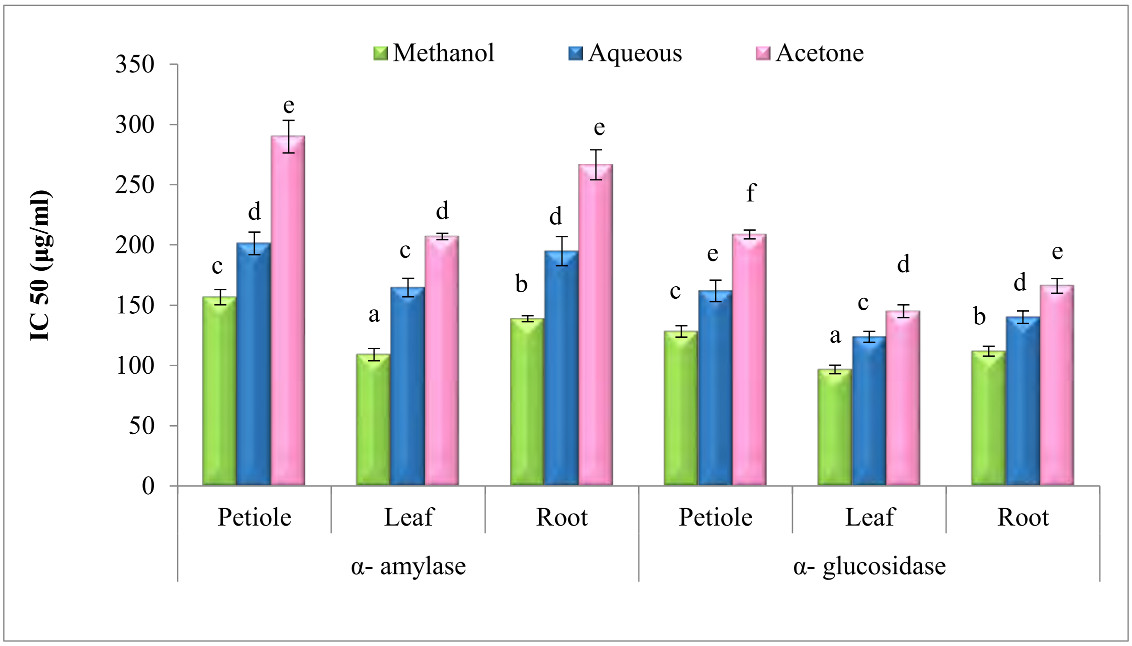


Fig 3. α-amylase and α- glucosidase inhibition activity of different parts of *Centella asiatica* obtained by various extraction solvents Results are represented as mean±SEM, n=3. Values with different letters (a, b and c) represent significant ($P< 0.05$) differences of means.

Secondary metabolites like phenolics and flavonoids present abundantly in plants, are considered as important dietary antioxidants that mitigate oxidative stress in biological systems (Tungmunnithum et al., 2018). The methanolic leaf extract had the highest total phenolic content (TPC) of 65.49 mg GAE/g DW and total flavonoid content (TFC) of 12.56 mg QE/g DW, surpassing petiole and root. Methanolic and aqueous extracts showed higher phenol and flavonoid content than acetone extracts, likely due to higher affinity for these solvents.

Qualitative analysis of phytochemicals in different solvent extracts of leaves, petiole and root revealed that methanolic extracts contained the most phytochemicals, followed by aqueous and acetone extracts. Alkaloids, saponins and tannins were present in all extracts, while glycosides were absent in the aqueous and acetone extracts of petiole and leaves and terpenoids were absent in aqueous extract (Table 3). The findings revealed that the distribution of bioactive compounds varies not only among different plants but also within different parts of the same plant. Such variation in the phytochemical profiling was reported by Salih et al., (2021), who parts and compounds.

concluded that solvent extraction varies with plant The efficacy of methanol as an extraction solvent was evident, enhancing the recovery of bioactive components from plant materials. Principal component analysis (PC1 78.60% and PC2 16.40%) confirmed this, showing the phytochemical attributes clustered with methanolic extraction (Figure 4). These findings align with those of Chigayo et al. (2016), who also reported highest antioxidant activity and elevated phenolics and flavonoids in methanol extracts of *Kirkia wilmsii* tubers. Furthermore, the present observation closely relates to the results obtained by Truong et al. (2019), who found methanol effective for extracting antioxidant compounds from *Severinia buxifolia*. Overall, *Centella asiatica* leaf extracts showed the most effective antioxidant activity and better phytochemical profiles compared to petiole and root, regardless of the solvent used. Methanolic extracts exhibited the best IC50 values against free radicals, α-amylase and α-glucosidase enzymes than distilled water and acetone extracts, likely due to higher phytochemical levels (Ghosal and Mandal, 2013). These results suggest that methanol is the optimal solvent for extracting bioactive compounds from *Centella asiatica* leaves.

Table 2. Quantitative analysis of phenol and flavonoid of different parts of *Centella asiatica* obtained by various extraction solvent**s.** Values represent mean ± SD (n = 3). Superscript letters within the same row indicate significant (P< 0.05) differences of means within the plant parts; Subscript letters within the same column indicate significant (P< 0.05) differences of means within the extraction

	TPC GAE mg/g DW			TFC QE mg/ g DW		
	Petiole	**Leaf**	**Root**	**Petiole**	**Leaf**	**Root**
Methanol	$43.35\pm2.15^{b}_{a}$	$65.49\pm3.46^{a}_{a}$	$43.35\pm2.33^{b}_{a}$	$7.95\pm0.48^{b}_{a}$	$12.56\pm0.67^{a}_{a}$	$3.72\pm0.22^{c}_{a}$
Aqueous	$29.16\pm1.42^{b}_{b}$	$39.76\pm2.12^{a}_{b}$	$21.81\pm2.04^{c}_{b}$	$4.82\pm0.36^{b}_{b}$	$9.22\pm0.29^{a}_{b}$	$2.08\pm0.19^{c}_{b}$
Acetone	$22.85\pm2.08^{b}_{c}$	$32.64\pm1.08^{a}_{c}$	$18.06\pm1.66^{c}_{c}$	$2.15\pm0.19^{b}_{c}$	$4.89\pm0.52^{a}_{c}$	$1.66\pm0.32^{c}_{b}$

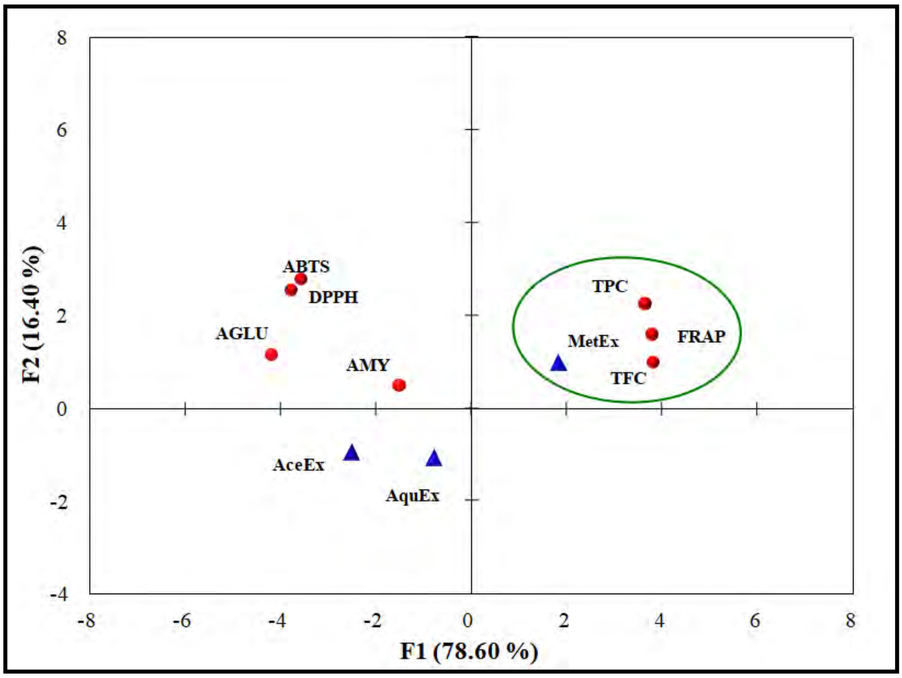
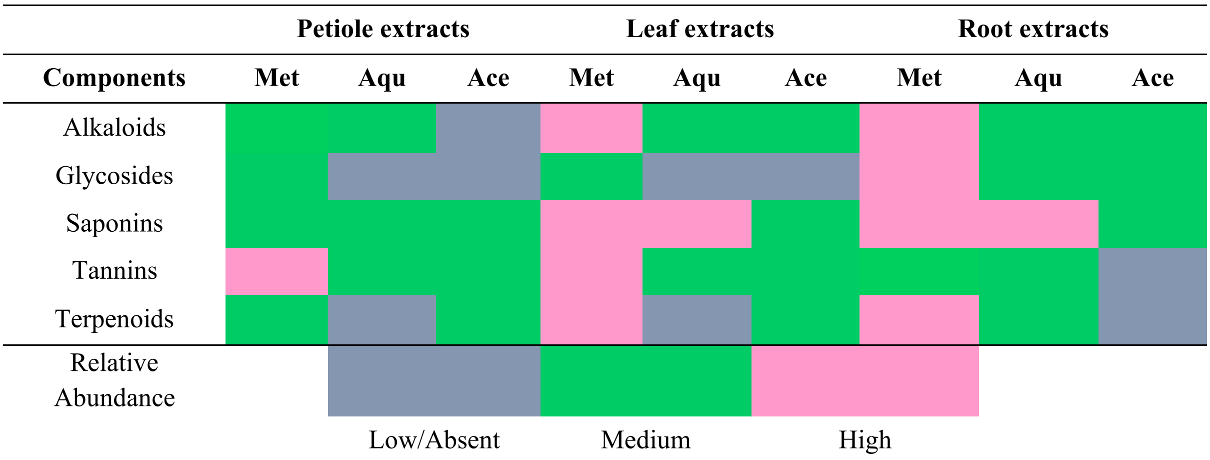


Fig 4. Principal component analysis of antioxidant, antidiabetic activities and associated phytochemical contents from *Centella asiatica* extracted by different solvents.

Table 3. Qualitative analysis of phytochemical constituents of different parts of *Centella asiatica* obtained by various extraction solvents



	Petiole extracts			Leaf extracts			Root extracts		
Components	**Met**	**Aqu**	**Ace**	**Met**	**Aqu**	**Ace**	**Met**	**Aqu**	**Ace**
Alkaloids	🟩	🟩	⬜	🟥	🟩	🟩	🟥	🟩	🟩
Glycosides	🟩	⬜	⬜	🟩	⬜	⬜	🟥	🟩	🟩
Saponins	🟩	🟩	🟩	🟥	🟥	🟩	🟥	🟥	🟩
Tannins	🟥	🟩	🟩	🟥	🟩	🟩	🟩	🟩	⬜
Terpenoids	🟩	⬜	🟩	🟥	⬜	🟩	🟥	🟩	⬜
Relative Abundance		⬜	⬜	🟩	🟩	🟥	🟥		
		Low/Absent		Medium		High			

Conclusions

This study investigated the extraction of *Centella asiatica* leaves, petiole and root using methanol, distilled water and acetone. Methanolic extracts contained the most phytochemicals, followed by aqueous and acetone extracts. Methanolic extracts showed the best IC50 values for DPPH, ABTS$^+$, as well as potential antidiabetic activity. Methanolic extract of *Centella asiatica* leaves was found to be a promising source for bioactive compounds with potential therapeutic property. Further research is suggested to identify individual bioactive components and explore their potential applications.

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