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Research Article

Evaluation of the Duration of Time and use of Different Solvents for Phytochemical Constituents, Antioxidants, and *In vitro* Anti-diabetic Activities of *Camellia sinensis* Leaves and Twigs

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ABSTRACT

Camellia sinensis is known to mankind centuries back, and the leaves are famous as tea leaves. The huge polyphenolics present in the aerial parts are the key factors for the health benefits. Our current study focussed on evaluating and comparing the influence of various solvents and duration of extraction on the secondary metabolites, antioxidant potential, and *in-vitro* anti-diabetic activity of the leaves and twigs of *C. sinensis.* Butanol and methanol were marked effective in the extraction to produce higher yields (45.66%) than other solvents. Duration of extraction also proved to influence the total yield; 48 hours is declared as an optimum duration of time to extract maximum contents from the plant material and proved better *in vitro* activities and phytoconstituents. Total phenolic and flavonoid contents were identified as higher in 48 hours, whereas total tannins were observed more in 24 hours. Antioxidant activity results performed through 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, phosphomolybdenum method and in vitro anti-diabetic activity results also supported the influence of extraction time and solvents.

Introduction

Tea is a renowned health drink made from the leaves and twigs of *C. sinensis* (Theaceae), an evergreen dicot plant. Though it is originated in China, it consumed worldwide and valued for its physiological and psychological benefits.[1] Ample literature on phytochemistry and pharmacology evident the importance of this herb in the management of stress and adaptogenic properties.[2-4] The high polyphenolic content in the aerial parts of tea leaves are responsible for the reported activities. The percentage and nature of the polyphenolics present in the tea leaves creates varieties of tea preparations such as green tea. Traditional systems of medicine such as *Ayurveda* and the Chinese system of medicine mentioned tea leaves for treating various ailments.[5] Tea leaves are pharmacologically proved to show a protective effect against obesity, osteoporosis, infections, and other metabolic syndromes.[6-9]

Extraction is the preliminary step in most plant-based research. The efficiency of extraction procedure depends on various factors such as method implied, polarity of solvents, and extraction time. The better extraction condition makes the extract obtained is rich with phytochemicals to have maximum pharmacological activity.[9] Extraction methods such as maceration, percolation, soxhlet, decoction and supercritical fluid extractions etc., are generally used; solvents such as petroleum ether, n-hexane, benzene, ethyl acetate,

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chloroform, n- butanol, methanol, ethanol, and water are the regular solvents used for the extraction.[10] Identifying an optimum condition for better yield and activity is a tedious process. The present study was carried out to compare the phytochemical, in vitro antioxidant and anti-diabetic activity of green tea made from leaves twigs. Also, a comparison was carried out between various hours of extraction.

Materials and Methods

Collection and Preparation of Extracts

Leaves and Twigs of *C. sinensis* were collected from Kannan Devan Tea Plantation, Munnar. Authentication was done by Professor Ravi Prasad, Department of Botany, SK University, Anantapur. The sequential extraction (Fig. 1) was carried out for the powdered material by maceration for 24, 48, and 72 hours with intermittent shaking by using various solvents viz., petroleum ether, n-hexane, n-butanol, ethyl acetate, methanol, water-based on their increasing polarity in the ratio of 1: 3 and percent yield was calculated.[11] Method and time of extraction were selected based on the previous studies performed elsewhere.[12]

Phytochemical Screening

Phytochemical screening of the extract was performed according to standard methods.[13]

Total Phenolic Content Estimation

The total phenolic content was estimated by the Folin Ciocalteu method as described by Singleton *et al.* (1965) with slight modifications. The absorbance was measured at 765 nm using a UV-vis spectrophotometer (Labindia 3000+). All the experiments were run in triplicate. The mean values and standard deviations were calculated.[14]

Total Flavonoid Content Estimation

The flavonoids content was determined by the method described by Chang *et al.* (2002). The absorbance was measured at 510 nm. The total flavonoids content was expressed as µg of quercetin equivalents per mg dry matter (µg QE/mg dry weight). All the experiments were run in triplicate. The mean values and standard deviations were calculated. [15]

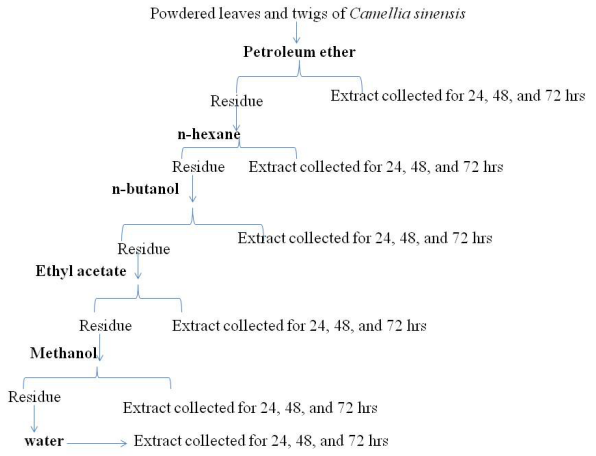

Fig. 1: Extraction procedure

Total Tannin Content Estimation

The tannin content was determined by the method of Broadhurst *et al*., 1978 with slight modification, using tannic acid as a reference compound and expressed as µg of tannic acid equivalents per mg dry matter (µg TAE/ mg dry weight). All the experiments were run in triplicate. The mean values and standard deviations were calculated.[16]

In vitro Antioxidant Activity (DPPH assay)

In vitro, antioxidant activity was determined by the method of Blois (1958), and the absorbance of the reaction mixture was measured in UV Visible spectrophotometer (Labindia 3000+) at 517 nm. Inhibition of DPPH radicals (%) was calculated, and IC_{50} value was determined.[17]

Total Antioxidant Activity

Total antioxidant capacity was determined by the phosphomolybdenum method, and absorbance of the mixture was measured at 695 nm by using UV Visible spectrophotometer (Labindia 3000+).[18]

In vitro Anti-diabetic Activity

This activity was performed according to the method described by Cirillo. Glucose was estimated, and absorbance was recorded using UV Visible spectrophotometer (Labindia 3000+) at 520 nm taking metformin as standard.[19]

Fractionation by Column Chromatography

Extracts collected at 48 hours were subjected to fractionation as they showed more in vitro antioxidant and anti-diabetic properties. Fractionation was carried out through column chromatography by using silica gel as a stationary phase. Stepwise elution was carried out using various eluents that is hexane, CCl_4, chloroform, ethyl acetate, methanol, and water.[20]

Results and Discussion

Extraction and Percentage Yield

Extraction of *C. sinensis* leaves, and sequentially twigs with various solvents lead to corresponding crude extracts and the solvent was removed using vacuum and the percentage yield was calculated (Table 1). The percentage yield was found to be high for twigs and extraction using n-butanol was identified as efficient, produced maximum crude mass with respect to time of extraction (Fig. 2).

Phytochemical Screening

The preliminary phytochemical investigation revealed that the extracts of *C. sinensis* are enriched with various

Table 1: Percentage yield of extracts *C. sinensis* with varying solvents and time

Extract	*24 hours*	*48 hours*	*72 hours*
C. sinensis leaf petroleum ether extract (CSLPE)	4.21	8.01	7.35
C. sinensis leaf n-hexane extract (CSLHE)	18.35	14.87	20.54
C. sinensis leaf n-butanol extract (CSLBE)	11.87	31.86	30.65
C. sinensis leaf ethylacetate extract (CSLEE)	10.35	12.41	13.54
C. sinensis leaf methanol extract (CSLME)	20.36	24.82	23.89
C. sinensis leaf aqueous extract (CSLAE)	14.25	13.25	13.98
C. sinensis twig petroleum ether extract (CSTPE)	4.96	4.29	6.52
C. sinensis twig n-hexane extract (CSTHE)	10.56	11.8	13.45
C. sinensis twig n-butanol extract (CSTBE)	35.65	42.25	45.66
C. sinensis twig ethylacetate extract (CSTEE)	13.98	12.68	11.28
C. sinensis twig methanol extract (CSTME)	19.27	22.47	23.05
C. sinensis twig aqeuous extract (CSTAE)	10.1	10.58	12.87

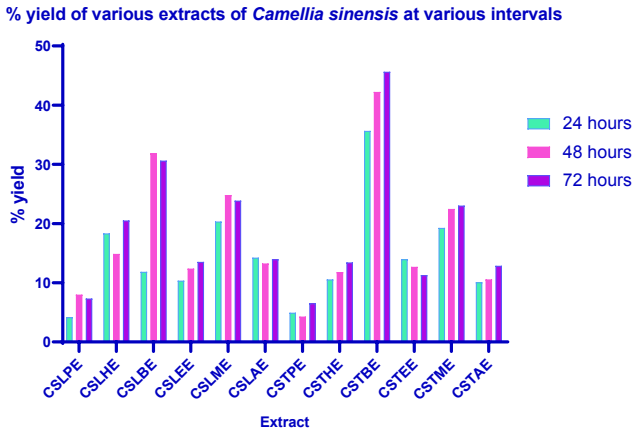

Fig. 2: Percentage yield of extracts *C. sinensis* with varying solvents and time

secondary metabolites such as Alkaloids, carbohydrates, flavonoids, phenols, steroids, terpenoids, glycosides, tannins, proteins, saponins. No difference was found in the time of extraction and phytochemicals present (Table 2).

Total Phenolic Content, Total flavonoid and Total Tannin Content

The total phenolic content, total flavonoid content, and total tannin content of the extracts were calculated and expressed as gram equivalents of gallic acid, Quercetin, and tannic acid, respectively (Figs 3-5). Results indicate that the extracts collected after 48 hours showed comparatively high total phenolic content and total flavonoid content than others, and CSTBE stood highest for both. But, total tannin content is observed in the extracts collected after 24 hours, and CSLME and CSLAE ranked high in the total tannin content among other fractions.

Table 2: Preliminary Phytochemical screening of leaves and twigs of Camellia sinensis

Phytochemical	*Present or Absent*
Alkaloids	+
Glycosides	+
Flavonoids	+
Terpenoids	+
steroids	+
Tannins	+
Proteins	+
Carbohydrates	+
Amino acids	+
Saponins	+

+ indicates the presence and – indicates the absence of phytochemicals

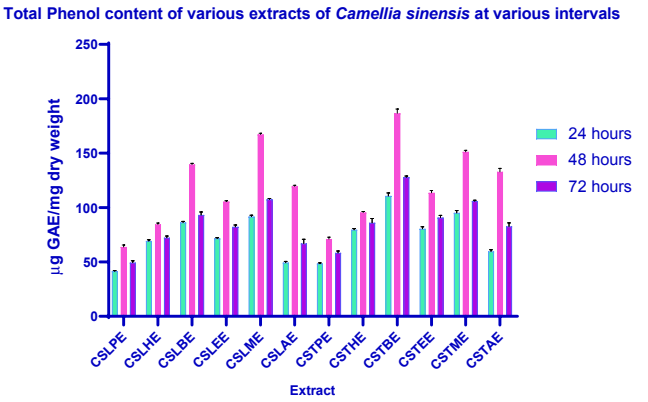

Fig. 3: Total phenolic content of various extracts of *C. sinensis*

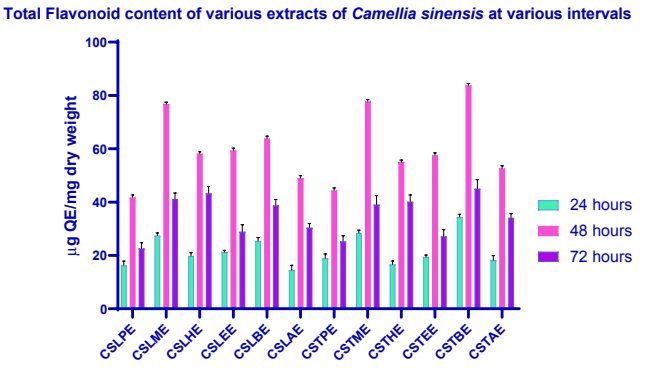

Fig. 4: Total flavonoid content of various extracts of ***C. sinensis***

In vitro Antioxidant Activity (*DPPH* assay)

Plants with polyphenolic compounds have been reported to possess free radical scavenging activity. All the extracts collected at regular intervals of time (24, 48, and 72 hours) were screened for their antioxidant activity by DPPH assay, and their IC_{50} values were calculated (Table 3 and Fig. 6). Results signify that extracts collected after 48 hours is showing excellent inhibition in a dose-dependant manner. Among the various extracts, the CSLME fraction showed excellent antioxidant activity when compared to standard Ascorbic acid.

From these results, it is clear that the extracts collected after 48 hours are showing better IC_{50} values. So, these extracts were fractionated using solvents with various polarity ranges and again screened for their antioxidant activity at various concentrations (25–200 µg/mL) through DPPH assay to identify the potent fraction (Figs. 7 and 8). Results showed that LM1, LM2, LM4, LE1, LH2, and LP3 are the fraction that is potent among other fractions of leaf extract. Similarly, TB1, TB2, and TM1 are the potent fractions of twig extracts collected after 48 hours of extraction.

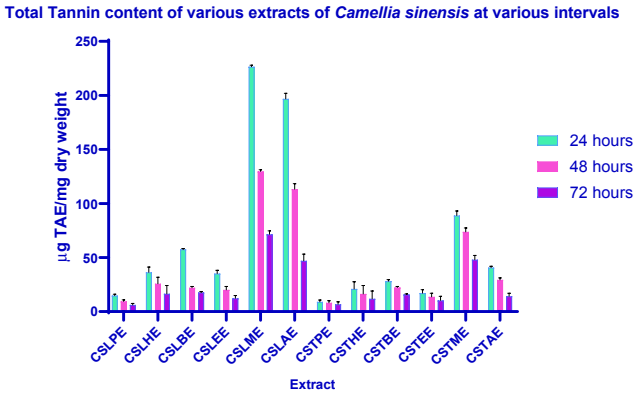

Fig. 5: The total tannin content of various extracts of *C. sinensis*

Table 3: DPPH Scavenging activity of *C. sinensis* extracted at various time intervals

	IC_{50}		
Extract	*24 hours*	*48 hours*	*72 hours*
AA	107.28	106.27	107.16
CSLPE	428.12	657.77	688.27
CSLHE	462.37	549.35	629.78
CSLBE	360.42	176.61	429.68
CSLEE	371.99	240.75	574.88
CSLME	221.71	113.47	417.42
CSLAE	306.31	221.6	502.51
CSTPE	513.91	565.13	681.57
CSTHE	488.27	505.28	610.03
CSTBE	358.22	114.33	423.28
CSTEE	418.925	249.14	553.93
CSTME	324.44	128.22	455.42
CSTAE	334.88	386.29	535.34

Total antioxidant activity by Phosphomolybdenum method

Total antioxidant activity of the leaves and twigs of *C. sinensis* collected at various time intervals were evaluated using phosphomolybdenum method. The extracts collected at 48 hours *C. sinensis* showed the highest total antioxidant activity among other extracts of the leaves, followed by the extract of twigs collected at 48 hours. This could due to the high contents of total phenolics and flavonoids in these extracts. When these active extracts are fractionated using various solvents followed by screening total antioxidant, fraction LM1, LH2, LM4, LE1, LM2, and LP3 showed better antioxidant activity than others. Similarly, TB1, TB2, and

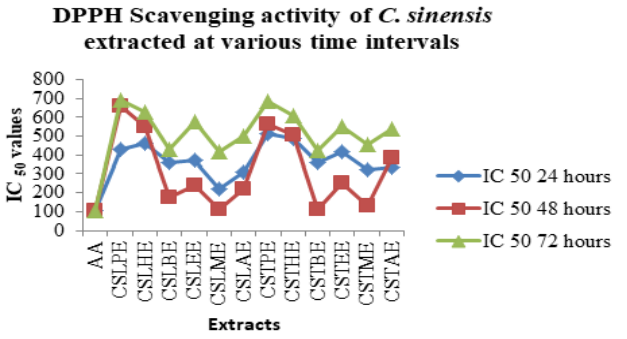

Fig. 6: DPPH Scavenging activity of ***C. sinensis*** extracted at various time intervals

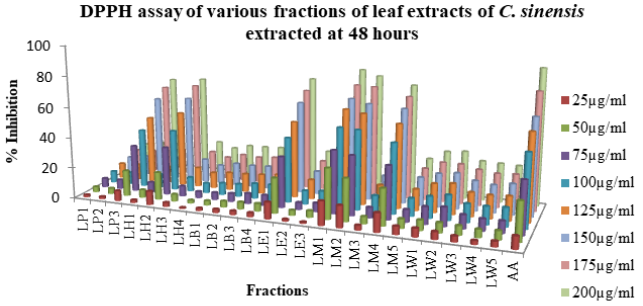

Fig. 7: DPPH assay of various fractions of leaf extracts of *C. sinensis* extracted at 48 hours

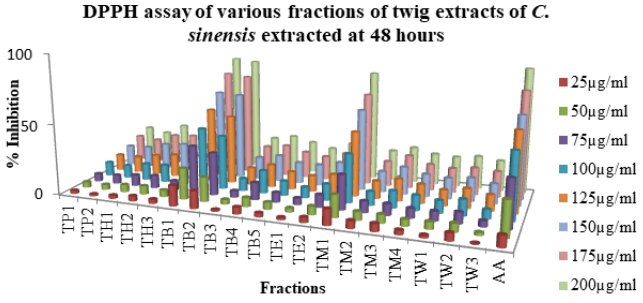

Fig. 8: DPPH assay of various fractions of twig extracts of *C. sinensis* extracted at 48 hours

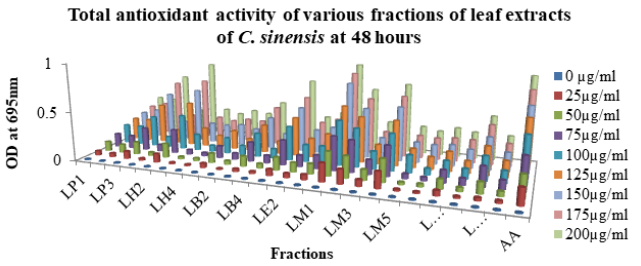

Fig. 9: Total antioxidant activity of various fractions of leaf extracts of ***C. sinensis*** extracted at 48 hours

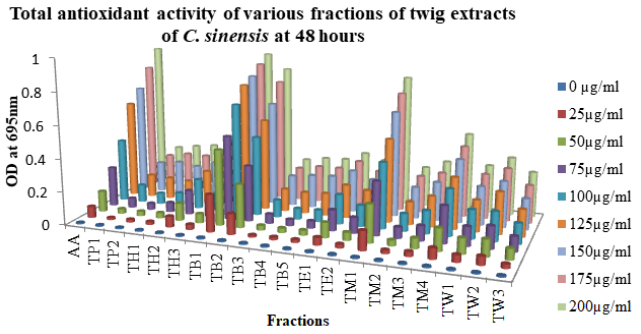

Fig. 10: Total antioxidant activity of various fractions of twig extracts of ***C. sinensis*** extracted at 48 hours

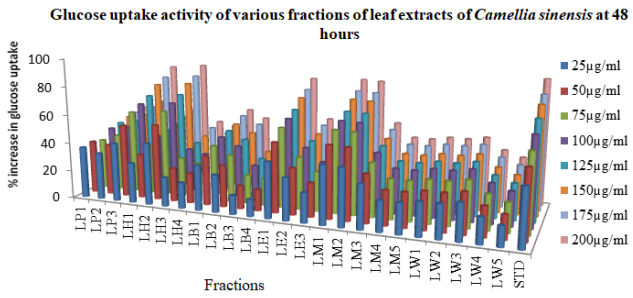

Fig. 11: Glucose uptake activity of various fractions of leaf extracts of ***C. sinensis*** at 48 hours

TM1 fractions of twigs showed the highest antioxidant activity. But, twigs showed comparatively better total antioxidant activity than leaves in a dose-dependant manner (Figs 9 and 10).

In vitro Anti-diabetic activity: Glucose Uptake by Yeast Cells

The extracts of *C. sinensis* collected at 48 hours promoted the uptake of glucose across the plasma membrane of yeast cells effectively. Fractions LH2, LM1, LP3, LM2, and LE1 of *C. sinensis* leaves promoted the glucose uptake efficiently than other fractions. Similarly, TB1 showed the highest among the fractions obtained from the *C. sinensis* twigs collected at 48 hours, followed by TB2, TH3, TM3, TE2, TH1, TM2, TM4, and TM1 fractions. The results concluded that the twigs showed better anti-diabetic activity than leaves (Figs 11 and12).

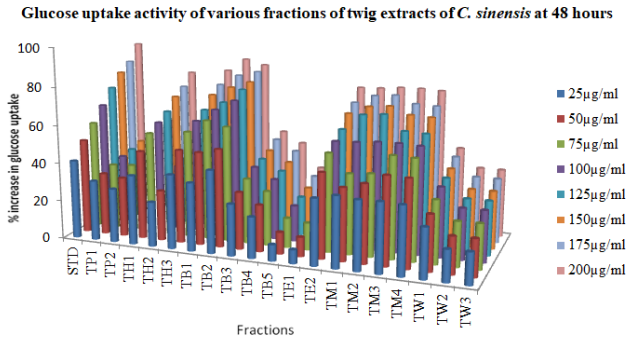

Fig 12: Glucose uptake activity of various fractions of twig extracts of *C. sinensis* at 48 hours

Conclusion

To conclude the results, twigs *C. sinensis* showed better antioxidant activity and anti-diabetic activity than leaves. Compared to all other extracts, extracts at 48 hours showed higher antioxidant activity and anti-diabetic activity when compared to that of 24 and 72 hours. Extracts collected at 48 hours were proved to be rich in total phenolic and total flavonoid content. Conversely, extracts collected at 24 hours showed higher total tannin content and no difference in phytochemical composition at different extraction intervals. From this study, it can be also be concluded that 48 hrs is the optimum extraction time to excerpt maximum polyphenolic contents both from the leaves and twigs of *C. sinensis.*

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