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Original Article

EXPLORATION OF BIOACTIVE COMPONENTS OF *THUNBERGIA COCCINEA*, ITS PHARMACOGNOSTIC, ANTIOXIDANT, GCMS AND ANTIHYPERGLYCEMIC STUDIES

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ABSTRACT

Objective: An effort currently made to appraise the preliminary phytochemical, pharmacognostic criteria, antioxidant, GCMS and antihyperglycemic investigations of the *Thunbergia coccinea* leaves. *Thunbergia coccinea (T. coccinea)* is an ornamental plant considerably practiced by the tribes of forest areas of Assam (INDIA) as an analgesic, antipyretic, anti-inflammatory, antidote, hepatoprotective, antidiabetic and detoxificant substance.

Methods: A comprehensive literature survey was conducted to recognize the ethnomedicinal value of *T. coccinea,* which is currently grown practically in all provinces. The physicochemical constants like moisture content, ash values especially total ash, insoluble acid ash, water-soluble ash and foreign organic matter were determined for the assessment of the drug. Pharmacognostic parameters like fluorescence examination and microscopic characters of the leaf were studied that would serve to verify for contamination. The extract secured by maceration was subjected to the phytochemical inquiry to determine the existence of substances and their antioxidant activity.

The antihyperglycemic characteristic of alcoholic extract of the leaf was examined with the inhibition of α-amylase and α-glucosidase enzymes. Gas Chromatography-Mass Spectrometry (GCMS) studies of alcoholic extract of the plant leaf have undertaken to get an insight into the therapeutic properties of the molecules present based on online PASS prediction.

Results: Various physicochemical, microscopic parameters studied gave a clear distinguishing and identifying features of *T. coccinea* leaf. Phytochemical screening gave an insight into the secondary metabolites existing in the plant leaf through picturizing its therapeutic properties against various ailments. Both extracts of *T. coccinea* leaf showed enhanced antioxidant activities.

Nevertheless, the alcoholic leaf extract has shown significant antioxidant activity with an IC_{50} of 171.38±2.51 µg/ml and AQTC an IC_{50} value of 206.29±4.5 µg/ml respectively by DPPH method. Further, ACTC showed a better-reducing potential with an IC_{50} value of 105.74±0.61 µg/ml in comparison with AQTC IC_{50} value of 203.702±0.97 µg/ml by FRP method. The inhibition potentiality of α-amylase and α-glucosidase was found to be 71.66 % and 83.74 %, respectively at 500 µg/ml that rationally an adequate remedy in the treatment of type-2 diabetes. GCMS studies of the alcoholic extract unveiled the presence of different molecules like Glycerol, tris (trimethylsilyl) ether, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Undecanoic acid, Ethyl ester, Phytol in comparison with NIST library, thereby giving its predicted therapeutic properties like sugar phosphatase inhibitor, antifungal, phobic disorders treatment, antiviral and so on.

Conclusion: The selected plant had many proven therapeutic traits and, possibly, successively united on to the sort of potential therapeutic plants. Besides, isolation and discoveries will lead to the detection of certain novel compounds, which will be of potential medicinal value.

Keywords: *Thunbergia coccinea*, Ethnomedicine, Pharmacognosy, Phytochemicals, Antioxidant assays, IC_{50} value, Antihyperglycemic, α-amylase, α-glucosidase, GCMS

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INTRODUCTION

Plantlife is the spectacularly vibrant contribution for humanity and is the source of diversity of medicines abreast numerous infirmities. Since 1981, 38 molecules have been derived from medicinal plants, out of which 1,130 new therapeutic agents have approved as pharmaceutical drugs. Synthetic drugs often embrace profoundly processed chemicals, which makes them harder to get processed and metabolized within a body which coaxes toxicity and adverse side effects. Plant molecules have clinically proven to be potent anticancer agents such as taxol isolated from *Taxus brevifolia* [1]. Extensive drug leads obtained from plants against diverse pharmacological aspirations such as vinblastine and vincristine, chemotherapeutic drugs originated from *Catharanthus roseus* [2], (+)- calanolide A, manifesting anti-HIV activity [3], Alzheimer's, diabetes, malaria, jaundice and so on [4]. There are 122 compounds identified with a defined structure obtained from 94 plant species, which are exercised as medicines globally, and 80 % of these have had an ethnomedicinal value alike or related to the common usage of the active ingredients of the plant [5].

Thunbergia is a category of flowering plants that appertain to the Acanthaceae family, native to tropical provinces of Africa, Madagascar, Australia, and South Asia. Remarkable kinds of *Thunbergia* are *Thunbergia laurifolia, Thunbergia coccinea, Thunbergia fragrans, Thunbergia erecta, Thunbergia grandiflora, Thunbergia mysorensis, Thunbergia annua, Thunbergia battiscombei, Thunbergia gregorii*, and many more which have numerous medicinal attributes [6-21]. *T. coccinea* is a tropical, ornamental, long-lived, perennial, flowering vine. It is a native of Assam, popularly known as Chongalota and is a rare plant [22, 23]. Various parts of *T. coccinea* are used as a vegetable, a health tonic, to treat the cuts and abrasions, and the leaf juice possesses antidote and hepatoprotective properties and used to treat diabetes, and eye infections. The leaf paste is devoured along with honey against cough, and the root is chewed to treat boils. Apart from these ethnomedicinal uses, prior articles have assessed anti-inflammatory, analgesic and antipyretic attributes of the methanolic essence of *Thunbergia coccinea* leaves in animal models [23-25].

Diabetes is an ailment that weakens the body's ability to process blood glucose. 90-95 % have type 2 diabetes, which is the most common ailment affecting around 29 million people in the US alone, which ensues due to insulin resistance [26]. American Diabetes Association pronounces diabetes as the seventh leading cause of

death in the US. The postprandial hyperglycemia in type-2 is primarily because of the upsurge in the concentration of free glucose in blood plasma, which is manageable. Nevertheless, its complications like retinopathy, neuropathy, nephropathy, cardiovascular diseases, Alzheimer's disease might set on with advanced stages and is fatal if not treated. In this reputation, herbal units are considered a better selection in the assessment of synthetics in the control of diabetes [27].

Our present work is intended to probe the preliminary phytochemicals, antioxidant inquiry with macerated extracts, GCMS study, and inhibitory potential of α-amylase as well as α-glucosidase of ethanolic extract, apart from the pharmacognostic studies of *Thunbergia coccinea* leaf that would serve as a marker to secure the plant as against adulteration. The bio-activity of this plant might lay a trampoline to scan for other prospects, which might strike for further discoveries conducive to an innovative breakthrough in the field of drug discovery.

MATERIALS AND METHODS

Abbreviations

AQTC-Aqueous extract of *T. coccinea*, ACTC-Alcoholic extract of *T. coccinea*, ROS-Reactive oxygen species, DPPH-2,2-Diphenyl-1-picryl-hydrazyl, FRP-Ferric ion reducing power, TPC-Total phenolic content, TAA-Total antioxidant assay, GCMS-Gas chromatography-mass spectrometry.

Chemicals

2,2-Diphenyl-1-picryl-hydrazyl (DPPH), gallic acid, 2-deoxy-D-ribose, ascorbic acid, α-amylase from porcine pancreas, *Saccharomyces cerevisiae* α-glucosidase, p-nitrophenyl-α-D-glucopyranoside was purchased from Sigma Aldrich Chemical Co, (St. Louis, MO, USA). Ferric chloride, phenanthroline, sodium dithionite and Folin Ciocalteu reagent were obtained from Merck Millipore (Darmstadt, Germany). Sodium carbonate, acetic acid, ammonia, ferric chloride, chloroform and ethanol was obtained from Merck India Ltd. (Mumbai, India). Dinitro salicylic acid, starch was obtained from Sisco research laboratories (Mumbai, India). All supplementary chemicals used in this learning were of analytical grade.

Authentication of the plant

T. coccinea was procured from a nursery in Madikeri, later it was grown in a private farm near H. D. Kote taluk, Mysore, Karnataka, its specimens were collected, mounted on an herbarium sheet for authentication by a taxonomist. The specimen was identified and authenticated as *Thunbergia coccinea* Wallich ex. D. Don of Acanthaceae by Dr. K P Sreenath, Professor of botany and a Taxonomist, Bangalore University, Bengaluru with the available floras and voucher specimens. A voucher specimen NRK 09 is preserved in the Department of Pharmacognosy, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Bannimantap, Mysuru-570 015, India for further references.

Preparation of plant leaf extract

The selected plant leaves were collected, washed, air-dried, minuted and stored in an airtight container. The extraction was carried out by the maceration process to prepare aqueous extract (AQTC), wherein a known quantity of dried leaf powder was placed in a closed vessel and water is added to soak the leaf powder. The set-up is left to stand for three days with occasional shaking. Sporadic shaking brings fresh menstruum to the particle for besides extraction. The solid residue (Marc) is squeezed to obtain the extract and filtered. The extract thus achieved was concentrated, filtered and dried on a water bath, scrapped and is stored in a vacuum desiccator at room temperature for further investigations.

The alcoholic extract is also prepared by maceration as stated above (ACTC), wherein a known quantity of dried leaf powder was placed in a closed vessel and alcohol is added to soak the leaf powder. The solid residue (Marc) is squeezed to obtain the solution after three days. The marc is subjected to multiple macerations with the fresh solvent to enhance the potency of extraction. The filtrate from multiple macerations blended collectively and the expressed liquids are mixed and clarified by filtration. The extract thus achieved was concentrated in Rota vapor, and dried on a water bath, scrapped and is stored in a vacuum desiccator at room temperature for further investigations.

Physicochemical and pharmacognostic parameters

The moisture content, ash value, insoluble acid ash, water-soluble ash, external organic matter has been performed and exhibited as table 1. The fluorescence analysis and powder microscopy characteristics were registered as specified in the standard procedures provided in 'The Ayurvedic Pharmacopoeia of India' [28, 29] and executed the qualitative phytochemical analysis.

Powder microscopy

The fine powder is stained with phloroglucinol+hydrochloric acid (HCl) and located on a slide with glycerine. Subsequent view beneath a microscope explicated the presences of the unicellular lignified trichomes, fibers, anomocytic stomata, calcium oxalate crystals, epidermal cells, and xylem vessels, etc.

Fluorescence analysis

Powdered leaf of *T. coccinea* was treated with various chemical reagents as mentioned in Table-2 and is exposed to visible and UV light to examine their fluorescent behavior. Fluorescence investigation of powdered leaf demonstrated beneficial to distinguish the powdered drug substance.

Phytochemical analysis

The preliminary phytochemical investigation was conducted with AQTC and ACTC to discern the presence of diverse constituents practicing conventional methods [29, 30].

Antioxidant studies

Free radical scavenging activity by DPPH method (DPPH)

Free radical scavenging effects of AQTC and ACTC were determined and compared with ascorbic acid, a standard compound using the DPPH method. The degree of discoloration due to the formation of reduced DPPH indicates the scavenging potentials of AQTC and ACTC. The change in the absorbance was recorded at 517 nm according to the method described by Nabanita Ghosh, Bhattacharya DK, 2020 [31], which is a measure of its antioxidant activity. Known antioxidant ascorbic acid (control) was taken in the range of 10 to 250 µg/ml. Working standards of AQTC and ACTC were prepared from the stock. All solutions were diluted with ethanol to final concentrations. To this 3 ml of ethanolic solution of DPPH was added to all dilutions, shaken well and the mixture was incubated at room temperature for 20 min. The control was prepared as above without the extracts. The readings were read at 517 nm using ethanol as blank.

Scavenging activity was expressed as the inhibition percentage calculated using the below formula,

$$\% \text{ Inhibition} = [\frac{(A_0 - A)}{A_0}] \times 100$$

Where A_0 is control absorbance, A is the sample absorbance.

Ferric ion reduction activity of the plant extract (FRP)

Antioxidants react with ferric ion (Fe^{3+}) to reduce and convert it to Fe^{2+}. Fe^{+2} rapidly reacts with 1, 10-phenanthroline and forms a red-colored, stable complex which has strong absorption in the visible spectrum at a wavelength of 510 nm. The degree of coloration indicates the reduction potential of the extracts. The reaction mixture containing AQTC and ACTC of different concentrations in the range of 20-200 µg in a final volume of 5 ml containing 0.2 mmol ferric chloride and 0.5 mg of O-phenanthroline was incubated for 15-20 min at ambient temperature. Reduction of sodium dithionite (0.3 mmol) assessed instead of the extracts and the absorbance is considered as equivalent to a 100 % reduction of all the ferric ions present [32]. Ascorbic acid was used as a standard. A graph is plotted, which depicts the Fe^{3+} reduction capacity of both AQTC and ACTC. IC_{50} values were calculated for both AQTC and ACTC and the standard compound.

Percentage inhibition (FRP) is calculated using the below formula

$$\% \text{ Inhibition} = [\frac{(A_0 - A)}{A_0}] \times 100$$

Where A_0 is the absorbance of the control and A is the sample absorbance

Total phenolic content of the leaf extract (TPC)

Phenolics are a class of phytochemicals that account for most of the antioxidant activity in plant products. Natural phenolic compounds from medicinal herbs include flavonoids, phenolic acids, tannins, coumarins, quinones and others. The Folin-Ciocalteu (FC) method works based on electron transfer and gives a measure of the reducing capacity of the phenolic content of the plant. The FC reagent, a mixture of tungstate and molybdates, relies on its reduction by the phenolic compound, which results in the formation of a blue coloured complex [33].

1 ug/ml plant extracts prepared with distilled water, 2 ml of 8 % sodium carbonate and 1 ml of freshly prepared FC reagent added and incubated the mixture at room temperature for 30 min. The quantified total phenolic content present in AQTC and ACTC was expressed as mg of gallic acid equivalent per g dry extract. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer.

Total antioxidant assay of the leaf extract (TAA)

Total antioxidant activity of ACTC and AQTC was estimated by phosphomolybdenum assay which was a measure of its free radical scavenging activity. Ascorbic acid was taken as the reference standard in quantifying the antioxidants present in the plant leaf extracts with the formation of phosphomolybdenum complex which measured its reducing power on the conversion of Mo^{6+} to Mo^{5+} in acidic medium [34].

The mixture of categorized concentrations of AQTC and ACTC plant leaf working standards with proper dilution and molybdenum reagent (0.6 M H_2SO_4, 28 mmol sodium phosphate, 4 mmol ammonium molybdate) was incubated at 95 °C for 90 min, cooled to room temperature, followed by measuring the optical density was read at 695 nm. Total antioxidant capacity was computed in terms of ug/mg ascorbic acid equivalent.

Antihyperglycemic activity

α-amylase inhibition assay

200 µl of different concentrations of plant extracts (ACTC) in the range of 100-500 µg was mixed with 200 ul of α-amylase (0.5 mg ml^{-1}) in sodium phosphate buffer, pH 6.9 containing 5 mmol/l NaCl and was incubated for 10 min at 30 °C. 200 µl of the starch solution (1 % in sodium phosphate buffer, pH 6.9) was added to each tube and was incubated for 3 min at room temperature.

The reaction was terminated by the addition of 200 µl dinitro salicylic acid reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 ml of 2 M NaOH and 20 ml of 96 mmol of 3, 5-dinitrosalicylic acid solution) and was boiled for 10 min in a water bath at 85–90 °C. The mixture was cooled to ambient temperature and was diluted with 5 ml of distilled water, and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. The blank with 100 % enzyme activity was prepared by replacing the extract with 200 µl of the buffer. The α-amylase inhibitory activity was expressed as percentage inhibition and was calculated using the equation given below. The % α-amylase inhibition was plotted against the extract concentration [35].

$$\% \, \alpha - \text{amylase inhibition} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

Inhibition assay for α-glucosidase activity

The reaction mixture comprising of varying concentrations of plant extract (ACTC) in the series of 100-500 µg premixed with 490 ul 0.1 M phosphate buffer (pH 6.8) and 250 ul of 5 mM p-nitrophenyl α-D-glucopyranoside. After preincubating at 37 °C for 5 min, 250 µl α-glucosidase was added and incubated at 37 °C for 15 min. The reaction was terminated by the addition of 2000 µl 0.2 M Na_2CO_3. The absorbance was determined spectrophotometrically at 400 nm on the UV-Vis spectrophotometer. The blank with 100 % enzyme activity was prepared by substituting the extract with 200 µl of the buffer. The % α-glucosidase inhibition was plotted against the extract concentration in a graph using Origin 8.5 [36].

$$\% \, \alpha - \text{glucosidase inhibition} = 100 \times \frac{(\text{Abs of control} - \text{Abs of sample})}{\text{Abs of control}}$$

GCMS analysis

GCMS examination conducted with GC Agilent 7890A with MS 5975C MSD that signifies a better resolution and minimum varied mass possibly with incredible sensibility. Mass spectrometer analyser employs electron impact ionization with a single Quadrupole. The evaluation of data for compound identification and quantification conducted exercising total ion count (TIC).

The experimental conditions

The DB 5 MS which was 30 ml x 0.25 mm ID x 0.25 um film thickness column practiced for analysis, and Helium used as a carrier gas with a constant flow rate of 1 ml/min. The inlet temperature maintained at 250 °C, and the flow Mode was splitless. The Mass range scan was 33 m/z–600 m/z, and the Polarity was $^{+}$ve.

Sample preparation

Methanol (1 ml) is added to the sample and 20 ul is taken in a glass vial for derivatization. The sample is dried out with pure N_2 gas, 40 ul of methoxyamine hydrochloride (20 mg/ml in pyridine) is added, and incubated for 1 h. The derivatizing reagent BSTFA: TMCS taken in the ratio of 99:1 added to the sample, and the mixture is incubated again for 60 min at 600 °C. The derivatized sample was diluted with methanol for 10 times and injected 1 ul of the resultant sample into the GCMS instrument. The initial temperature of 45 °C/min with a hold time of 1 min, and a total time of 1 min and the ramp maintained at 290 °C/min, rate of flow being 8 °C/min with a hold time of 4 min and a total time of 37.6 min.

Identification of photo components

Interpretation of GCMS mass-spectrum was conducted using the National Institute of Standards and Technology (NIST 2011) chemistry web book comprising 243,893 spectra of 212,961 different chemical compounds [37]. The spectrum of the unknown substance compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials ascertained. The recognized compound bioactivities were predicted based on online PASS prediction.

RESULTS

The yield was determined to be 19.136 % w/w for AQTC and 16.8 % w/w for ACTC. Slight better yield was recorded with AQTC in comparison with ACTC.

Physicochemical parameters

Plants have active therapeutic ingredients, which commonly necessitates a complex mixture of phytochemical constituents that promotes a big deal with their quality assurance. Identity, purity, safety and quality are the essential parameters for standardizing herbal drugs. Physicochemical parameters play an indispensable role in the standardization of crude drugs. The studies such as moisture content represent the water percentage and the volatile matter present in the plant leaf powder that would deteriorate quickly. Ash value is a measure of inorganic mineral content such as K, Mg, Ca present in the drug/plant powder. High acid insoluble ash indicates the presence of salicaceous substances. % w/w±SD values of different physicochemical parameters are recorded as above in table 1.

Table 1: General physicochemical parameters of *T. coccinea* leaf

S. No.	Physicochemical parameter	% w/w±SD*
1.	Moisture content	6.14±1.9
2.	Total ash value	3.6±0.04
3.	Acid insoluble ash	1.82±0.02
4.	Water-soluble ash	1.76±0.06
5.	Foreign matter	0.51±0.08

*SD-Standard deviation (n=3)

Pharmacognostic parameters

Powder microscopy and fluorescence analysis of plant leaf

The detailed powder microscopy of plant leaf reveals the presence of various morphological and anatomical structures as witnessed in fig. 1. The fluorescence analysis of leaf powder treated with various reagents is summarised in table 2 to register the characteristics of the crude drug.

Although these methods are cost-effective, simple, they are much imperative and reliable to create correct identification, standardization, authentication and purity of the drug. These pieces of evidence will also be helpful to differentiate the plant from other closely related species of the same family. These characteristics serve a vital role before evaluating the medicinal traits of a plant thereby pledging its authenticity.

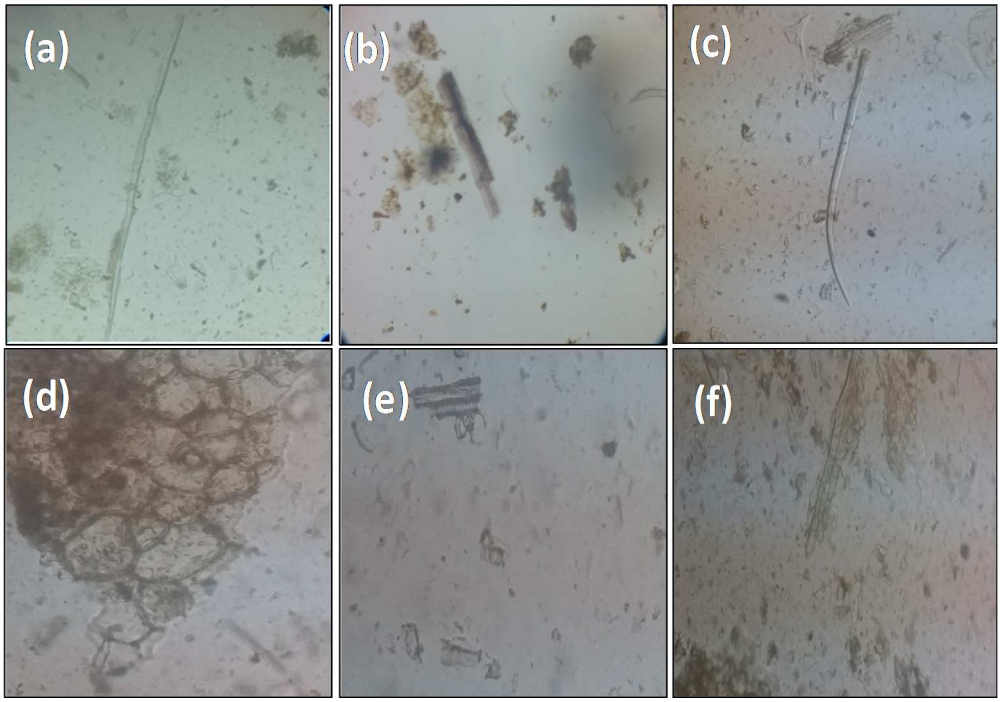


Fig. 1: Powder microscopy showing the presence of a) fibres b) medullary rays c) unicellular lignified trichomes and fibres d) parenchymatous cells e) vascular bundles f) xylem vessels

Table 2: Fluorescence characteristics of leaves of *T. coccinea*

S. No.	Particulars of the treatment	Under normal light	Under UV light (366 nm)
01	Powder as such	Green	Brown
02	Powder+1N NaOH (aqueous)	Dark green	Black
03	Powder+1N NaOH (alcoholic)	Green	Brown
04	Powder+1N HCl	Green	Black
05	Powder+H_2SO_4 (1:1)	Dark green	Brown
06	Powder+HNO_3 (1:1)	Brown	Black
07	Powder+Ammonia	Green	Black
08	Powder+Iodine	Reddish-brown	Green
09	Powder+5% $FeCl_3$	Green	Dark brown
10	Powder+Acetic acid	Brown	Black

Phytochemical analysis

Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, tannins, flavonoids, and lactones in AQTC. Sterols, alkaloids, carbohydrates, tannins, and lactones were present in ACTC (table 3). The presence of these secondary metabolites in *T. coccinea* is reported to confer therapeutic and biological properties.

Table 3: Qualitative analysis of phytochemicals of *T. coccinea* leaf elixir

S. No.	Metabolite	Tests/Reagent	AQTC	ACTC
1.	Sterols	A) Salkowski test	-	+
		B) Liebermann-Burchard test	-	+
2.	Tri-terpenes	A) Salkowski test	-	-
		B) Liebermann-Burchard test	-	-
3.	Saponins	A) Foam test	-	-
		B) Hemolysis test	-	-
4.	Alkaloids	A) Wagner's test	-	+
		B) Mayer's test	-	+
		C) Dragendorff's test	-	+
		D) Hager's test	-	+
5.	Carbohydrates	A) Molisch's test	+	+
		B) Fehling's test	+	+
6.	Tannins	A) Ferric chloride test	+	+
		B) Gelatin test	-	-
		C) Vanillin-HCl test	+	+
		D) Match stick test	+	+
7.	Flavonoids	A) Shinoda test	-	-
		B) Ferric chloride test	+	+
		C) Lead acetate test	+	+
		D) Zinc-hydrochloride acid reduction test	-	-
		E) NaOH test	+	-
8.	Lactones	A) Legal test	+	+
		B) Baljet test	+	+

('+' indicates presence and '–' denotes absence)

Antioxidant assays

Antioxidants are molecules comprehended to prevent the oxidative damage by neutralizing the ROS, thereby impeding the destruction indicated to the cells of organisms. The non-enzymatic, natural antioxidants include carotenoids, thiols, tocopherols, ascorbic acid, flavonoids and tannins that are attained from natural plant sources. A wide variety of antioxidants from both natural and synthetic origin have been proposed for use in the treatment of several human infirmities mainly related to heart, neurodegenerative diseases.

Some synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ) are generally used in processed foods. However, these compounds have said to shown toxic effects like liver impairment mutagenesis and as carcinogens. Flavonoids and other phenolic compounds of plant origin are engraved as scavengers of ROS. The function of the antioxidant system is to keep them at an optimum level. Hence, the present study was initiated to examine the antioxidant potential of AQTC and ACTC on distinctive *in vitro* models.

Free radical scavenging by DPPH method

DPPH radical is widely practiced for measuring the scavenging power of free radicals of phytoconstituents. Reaction mixture along with DPPH was incubated with increasing concentrations of *Thunbergia coccinea* leaf extracts experimentally and % inhibition is calculated in comparison with the control (without extract). Further on, the IC_{50} value was also calculated for the fractions as well as the standard by subjecting the data of % inhibition to OriginPro 8.5 analysis. The results showed a dose-dependent increase in the scavenging potential of AQTC and ACTC. On relative comparison of both the fractions, ACTC showed better DPPH radical scavenging ability with an IC_{50} of 171.38±2.51 µg/ml and AQTC an IC_{50} value of 206.29±4.5 µg/ml respectively. The IC_{50} of reference standard ascorbic acid was 6.56±0.54 µg/ml (fig. 2).

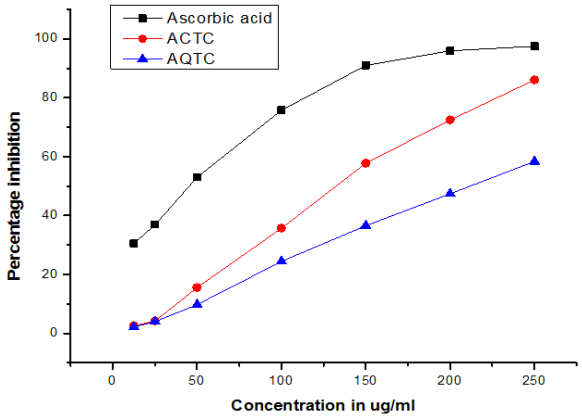


Fig. 2: DPPH radical scavenging activity of different fractions of *Thunbergia coccinea*

Ferric ion reduction activity

The investigative study of macerated leaf extracts of *T. Coccinea* reveals high reducing power. The reducing capacity of a compound is related to the electron relocation capability of that compound. An increase in the absorbance of the reaction mixture indicates an increase in the antioxidant activity.

Previous studies have already reported potent antioxidant activity of methanolic extracts of *T. coccinea* [25]. Present

studies revealed a higher reducing power in ACTC than AQTC. ACTC showed a better-reducing capability with an IC_{50} value of 105.74±0.61 ug/ml and AQTC an IC_{50} value of 203.702±0.97 ug/ml (fig. 3).

Total phenolic content

The outcome of the present investigation revealed a considerable range of TPC among AQTC and ACTC. The TPC in *T. coccinea* leaf extracts examined in this survey is summarised in table 4, which is numerically expressed as mg gallic acid equivalent (GAE) per gram of dried plant sample.

Gallic acid was used as a reference to plot the standard graph in the folin ciocalteau method. Analysis of the extracts showed a significantly high range of polyphenolics in ACTC comparatively.

Total antioxidant assay

The totality of antioxidants was quantified via the phosphomolybdate method in the leaf extracts of *T. coccinea,* which is shown in fig. 4. There was considered significant inhibition of free radicals of phosphomolybdate from $Mo^{6+}$to Mo^{5+}, which corresponds to the reducing power of the plant extracts in increasing concentrations. Here, the reducing power on phosphomolybdate was pointedly high in ACTC fraction and less in AQTC with standard ascorbic acid.

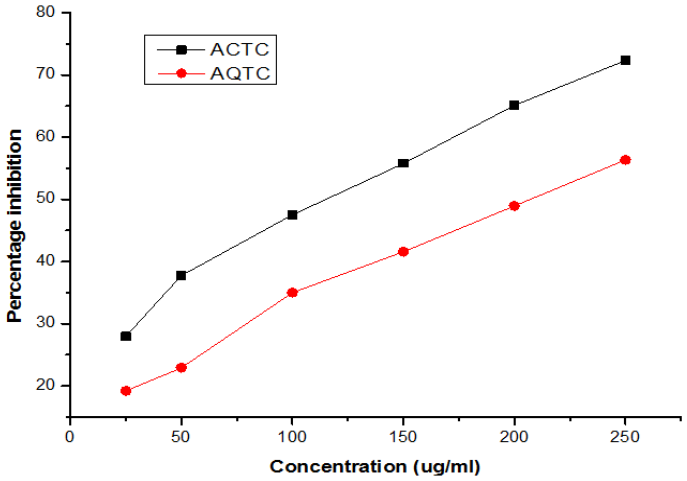


Fig. 3: Ferric ion reduction activity of AQTC and ACTC

Table 4: Quantification of phenolic compounds with antioxidant properties from AQTC and ACTC

Samples	TPC*
AQTC	216±0.60
ACTC	250±8.13

*TPC is expressed in Gallic acid equivalent value in mg/g of extract, Results were expressed as mean±SD of triplicate determinations.

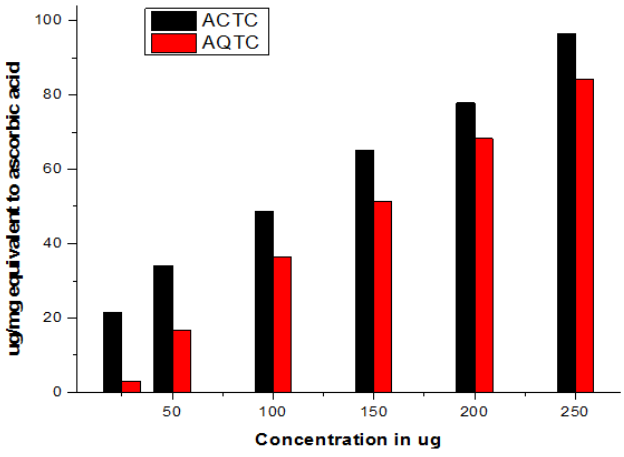


Fig. 4: Total antioxidant capacity of AQTC and ACTC

Table 5: IC_{50} values for AQTC and ACTC for different free radical scavenging assays

Samples	DPPH	FRP
AQTC	206.29±4.5[a]	203.702±0.97[a]
ACTC	171.38±2.51[a]	105.74±0.61[a]

IC_{50} (µg/ml) represents the effective inhibition concentration at which 50 % of the respective free radicals are scavenged, [a]Values are in ug/ml

Antidiabetic activity

α-amylase and α-glucosidase inhibition assays

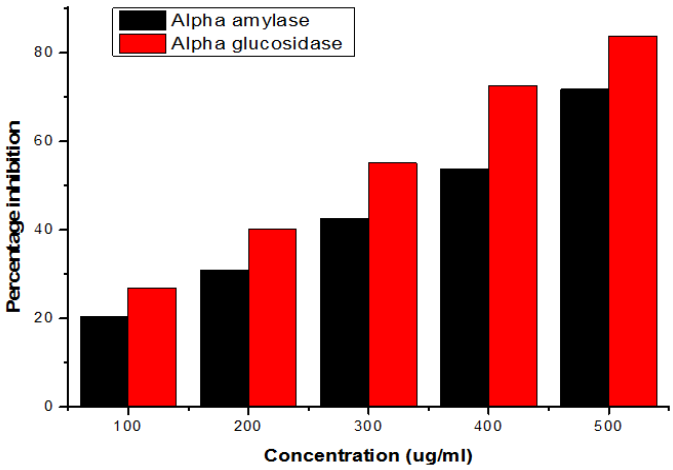


Fig. 5: α-amylase and α-glucosidase inhibition assays at varying concentrations of ACTC in the range of 100-500 ug comparatively

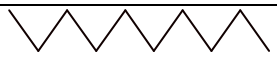
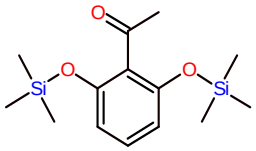
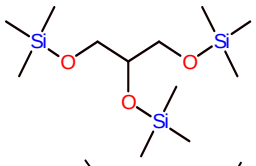
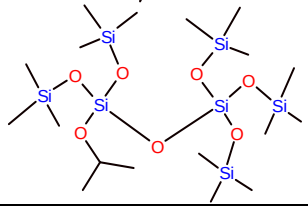
Antioxidant-rich ACTC extract of *T. coccinea* leaf showed very good α-amylase and α-glucosidase inhibition activity which is evident from the studies (fig. 5). The inhibition activity of α-amylase and α-glucosidase of ACTC increases with increasing concentrations of plant leaf extracts. The inhibition potentiality of α-amylase and α-glucosidase of ACTC was found to be 71.66 % and 83.74 %, respectively, at 500 µg/ml and thereby justifying the ethnomedical use of the plant.

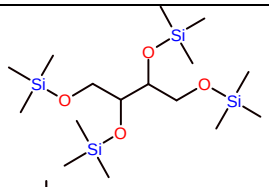
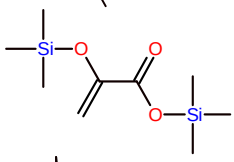
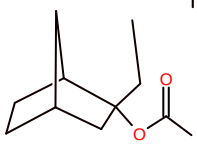
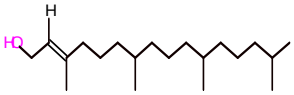
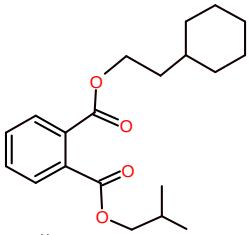
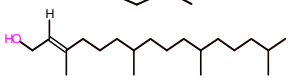
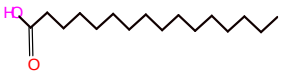
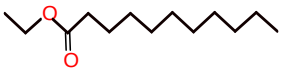
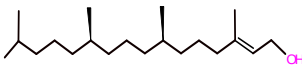
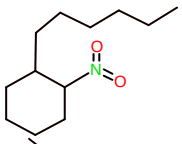
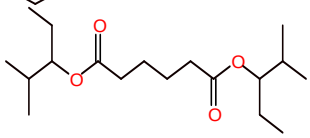
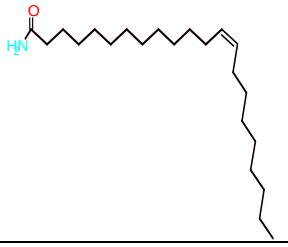
GCMS analysis

The alcoholic extract of the plant leaf contains both polar and non-polar components. The macerated ACTC on GCMS analysis helps in the identification of the molecules. The mass spectrum authenticated the existence of various components with different retention times (RT) (fig. 5). Different compounds elute out at different RTs which are analysed by MS to identify the nature and structure of the compounds. The large compound fragments into small components giving rise to the appearance of peaks at different m/z ratios. These mass spectra are the fingerprint of that compound, which can be identified by comparing it with the data from the NIST library.

TIC of GCMS and its comparison with NIST authorizes the presence of various components as mentioned in table 6. The major compound present in ACTC was Glycerol, tris (trimethylsilyl) ether with the existence of 29.877 % with an RT of 14.21 min. The subsequent abundant component was 2-Propenoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester with an RT of 21.161 min and 22.16 % existence. Next, in turn, was 3,7,11,15-Tetramethyl-2-hexadecen-1-ol with an RT of 22.958 min and 7.304 % existence and n-Hexadecanoic acid with an RT of 24.5099 min and an existence of 6.10 %.

Table 6: Compounds identified in ACTC through GCMS

RT (min)	Phytochemical compound [a]	Molecular formula	Molecular weight	Structure	Biological activities [b]
8.7479	Decane	$C_{10}H_{22}$	142.286		Inhibitor of sugar phosphatase, treatment of phobic disorders, anti-eczematic
12.204	2',6'-dihydroxyacetophenone, bis(trimethylsilyl) ether	$C_{14}H_{24}O_3Si_2$	296.513		Antineoplastic (colorectal and colon cancer), stimulant of erythropoiesis, antiosteoporotic, treatment of bone diseases, antifungal, carminative
14.215	Glycerol, tris (trimethylsilyl) ether	$C_{12}H_{32}O_3Si_3$	308.6372		Antineoplastic, inhibitor of sugar phosphatase, macrophage colony-stimulating factor agonist, an inhibitor of HIV-1 reverse transcriptase
17.5	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	$C_{18}H_{52}O_7Si_7$	576.20979		Inhibitor of sugar phosphatase, antineoplastic, an inhibitor of complement factor D

18.121	meso-Erythritol, tetrakis(trimethylsilyl) ether	$C_{16}H_{42}O_4Si_4$	410.8443		Inhibitor of sugar phosphatase, antineoplastic, an inhibitor of complement factor D, treatment of phobic disorders
21.161	2-Propenoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_9H_{20}O_3Si_2$	232.4243		Antineoplastic, inhibitor of angiogenesis, an inhibitor of HIV-1 reverse transcriptase, an inhibitor of complement factor D
22.117	2-Ethyl-2-norbornyl acetate	$C_{11}H_{18}O_2$	182.13068		**
22.9583	3,7,11,15-Tetramethyl-2-hexadecane-1-ol	$C_{20}H_{40}O$	296.539		Treatment of phobic disorders, an inhibitor of sugar phosphatase, anti ulcerative, antiviral (rhinovirus), macrophage colony-stimulating factor agonist, stimulant of platelet aggregation, antifungal
23.2796	Phthalic acid, 2-cyclohexyl ethyl isobutyl ester	$C_{20}H_{28}O_4$	332.19876		Inhibitor of sugar phosphatase, fibrinolytic, treatment of phobic disorders, general anesthetic, antiviral (rhinovirus), a vasodilator (peripheral)
23.5244	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.539		Protector of mucomembrane, treatment of phobic disorder, an inhibitor of sugar phosphatase, anti ulcerative, macrophage colony-stimulating factor agonist, antiviral (rhinovirus)
24.5099	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43		Protector of mucomembrane, treatment of phobic disorders, antieczematic, macrophage colony-stimulating factor agonist
24.9571	Undecanoic acid, ethyl ester	$C_{13}H_{26}O_2$	214.3443		Inhibitor of sugar phosphatase, antieczematic, treatment of phobic disorders
26.3414	Phytol	$C_{20}H_{40}O$	296.5310		Antihelmintic, antibacterial, anti-inflammatory, antioxidant, anticarcinogenic, antimicrobial, antinociceptive
26.6359	1-Hexyl-2-nitrocyclohexane	$C_{12}H_{23}NO_2$	213.172879		Glycopeptide-like antibiotic, antiviral (picornavirus), treatment of phobic disorders, an inhibitor of sugar phosphatase, fibrinolytic, vaso protector, anti-inflammatory
26.9095	Adipic acid, di(2-methylpent-3-yl) ester	$C_{18}H_{34}O_4$	314.24571		**
33.183	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337.5829		Macrophage colony-stimulating factor agonist, antieczematic, protector of mucomembrane, treatment of phobic disorders, stimulant of platelet aggregation, scavenger of oxygen

[a]Compounds were acknowledged referring to NIST 2011 library, [b]Actions were acknowledged based on online PASS prediction, **Activity not stated

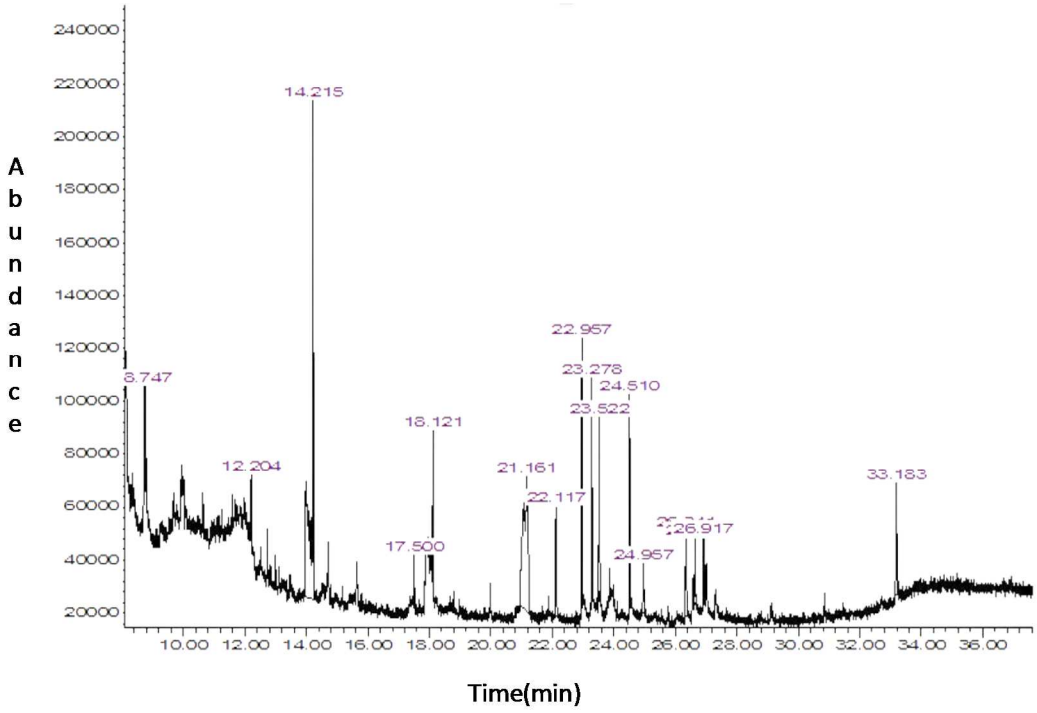


Fig. 6: GC-MS spectrum of various components of ACTC with different retention times

DISCUSSION

As there is no evidence for physicochemical, qualitative phytochemical assessment of *T. coccinea* leaf, its GCMS studies, its identifying and distinguishing studies of the plant drug from other species through microscopic characters, antioxidant activity with macerated aqueous and ethanolic extract, antihyperglycemic studies, present work is taken up to standardize the *T. coccinea* extracts as per World Health Organization (WHO) guidelines, and standard laboratory procedures to reach the pharmaceutical entrepreneurs and academicians. The methanolic extract of the *T. coccinea* leaf has asserted for therapeutic properties especially the analgesic, antipyretic, anti-inflammatory, antioxidant and further scrutinized in animal models [38]. The consequences ascertained the potent antioxidant potential with macerated-aqueous and alcoholic distillations. Better antioxidant potential is observed in ACTC with all assays in comparison with AQTC and the standard compound. These antioxidants administer a crucial role in guarding our body against numerous infirmities, which have shielding impacts on the free radicals released in our body. Relatively exceptional reduction at a very lower concentration indicates their high antioxidant potential. Investigations verified the concrete direct correlation between antioxidant activity and the concentrations of AQTC and ACTC before the reach of the threshold level. The studies are in correlation with the antioxidant studies done on *T. coccinea* leaf earlier on methanolic extracts.

Inhibition of α-amylase and α-glucosidase enzymes linger the breaking down of carbohydrates, thereby reducing increased postprandial blood glucose in diabetics. This investigation complies with the ethnomedicinal utilization of the plant leaf *T. coccinea* and possibly an efficient remedy for type-2 diabetes, wherein an increase in blood glucose leads to several complications and ailments. Alcoholic extract of *T. coccinea* leaf showed good inhibition of α-amylase and α-glucosidase, which might owe to the presence of high antioxidant content and various phytochemicals. Further on, *in vivo* studies on this aspect is to be conducted.

Alcohol is slightly less polar than water and dissolves more of oxidized aromatic constituents leaving behind carbohydrates and waxes. It is a better solvent of organic molecules and isolates secondary plant metabolites. However, further studies should be concentrated on looking for isolation and identification of secondary metabolites from ACTC, which shows better antioxidant activity.

GCMS analysis discloses the type of molecule present in the extract of the given plant *T. coccinea*, considerably aiding to concede the underlying mechanism of performance of such molecules in therapeutic properties. GCMS exposed the presence of around 5-6 silicon-based compounds in ACTC. However, studies have proved the access of many organosilicon compounds into clinical studies, which are silperisone, zifrosilone and many more. Reports also confirm that number of toxicology studies on organosilicon compounds is less than the number of its pharmacological actions [39]. The investigation of these compounds from ACTC of *T. coccinea* leaf might open a new venture for the discovery of novel compounds. However, this study also substantiates its use as a drug confirming the presence of various other phytochemicals too, having therapeutic properties. The classified compounds by GCMS possess various therapeutic properties such as antidiabetic, vasoprotective, anticancerous, antiviral, eczematic, antifungal, antimicrobial, stimulation of the immune system, stimulation of aggregation of platelets, and the activities of which were acknowledged based on online PASS prediction.

This study satisfies the standardization procedures, provides scientific proof of healing property of *T. coccinea,* which is in use by the tribes as an ethnobotanical medicine for a long time, giving a comprehensive perspective of the plant to establish itself as herbal medicine.

CONCLUSION

The variable ethnomedicinal applicability encapsulates the numerous healings and biological characteristics of the plant, have demonstrated scientifically by the study on its phytochemicals, and therapeutic features. However, the earlier studies on different parts of this medicinal plant are yet to be questing to examine. Nevertheless, with a basic idea of a distinct important class of molecules present in the plant extract, the synergistic effect of numerous secondary metabolites and their interdependence on each other is to be looked for in conjunction with their enzymatic systems. Furthermore, future research to be focused on the clinical activities of the drug lead, its efficacy, and toxicity can be taken up further on for animal studies.

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AUTHORS CONTRIBUTIONS

Kokila N. R.: The concept, design and execution of the experiments, acquisition of data and drafting of the manuscript, Dr. Mahesh B.: Conceptualization, design of the study, verification, interpretation of the acquired data, editing and reviewing of the manuscript, Dr. Mruthunjaya K.: Conceptualization, interpretation of the acquired data, reviewing of the manuscript.

CONFLICT OF INTERESTS

There are no conflicts of interest

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