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EVALUATION OF PHYTOCHEMICAL ANALYSIS, METAL ION CONTENTS, IN CORRELATION TO *IN VITRO* ANTIOXIDANT ACTIVITY OF *FICUS ELASTICA* AND *CYAMOPSIS TETRAGONOLOBA*

Manish Kaushik[1,2], *Akash Jain*[3*]

Address(es):
[1] Research Scholar, M.M. College of Pharmacy, M.M.(Deemed to be University), Mullana, Haryana, India-133207.Orcid ID: 0000-0002-1691-4187.
[2] Assoc. Professor, SBS College of Pharmacy, Palampur, Himachal Pradesh-176061, India.
[3] Professor, M.M. College of Pharmacy, M.M.(Deemed to be University), Mullana, Haryana, India-133207.Orcid ID: 0000-0001-5703-6117.

*Corresponding author: akash2911@gmail.com; manishkaushik63@gmail.com

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ABSTRACT

The present study was aimed with the identification of potent active phytochemicals followed by HPLC determination of presence of required bioactive components in *Ficus elastica* and *Cyamopsis tetragonoloba*. Thereafter, total phenolics were estimated and compared based on phytochemical screening. Determination of metal ions from the leaves of both plants was estimated by Atomic Absorption Spectrophotometer and finally, results were correlated with the potent antioxidant activity. DPPH, and Hydrogen per oxide scavenging assay methods were carried out for the antioxidant assay.IC50 value was determined as 3.45 and 134.78 µg/ml for CT extracts in DPPH and H2O2 assays respectively. Phytochemical analysis showed presence of alkaloids, glycosides, flavonoids, phenolics, tannins, terpenoids, and sterols in *Ficus elastica* leaves and alkaloids, glycosides, flavonoids, phenolics, and sterols in *Cyamopsis tetragonoloba* beans when various chemical tests were performed. TLC was identified various alkaloids and flavonoids from both the plants. Presence of *beta*- sitosterol and lupeol were identified and estimated by HPLC method (3.41 and 5.37 mg *beta* sitosterol and lupeol respectively in CT extract and 6.48 and 3.01 mg *beta* sitosterol and lupeol respectively in FE extract). Metal ions viz. Fe, Mg, Cu and Zn were identified as essential heavy metals with very negligible non essential heavy metals viz. Cd, Ni, As and Pb were estimated from both the plants whereas, Na, Ca, and K were higher in both of them. Finally correlation study among the metal ions, total phenolics, with antioxidant potentiality was established and confirmed the role of plant constituents in effective said activity.

Keywords: Analysis, Atomic Absorption Spectrophotometer, *Cyamopsis tetragonoloba*, *Ficus elastica*, HPLC, metal ions

INTRODUCTION

It is known to all that, Oxidative stress, which is brought on by excessive ROS production, can result in a variety of illnesses. Under these circumstances, the body needs the help of antioxidant-rich diet to counteract free radicals and undo the damaging effects of oxidative stress. Medicinal herbs, fruits, vegetables, cereals, and other foods are known to contain a variety of phenolic compounds with potent antioxidant properties. There is a strong correlation between these substances and their capacity as antioxidants (**Tripathi *et al.*, 2007**). The use of natural antioxidants derived from plant extracts or isolated plant products is on the rise in substitute of synthetic antioxidants, which raise safety concerns. Therefore, the present study was selected fortwo different plants but they both generate latex, such plants were*Cyamopsis tetragonoloba* and *Ficus elastica*.

Cyamopsis tetragonoloba (L.) Taub. (CT) is commonly known as Guar bean (F: Leguminosae). The plant is annually grown with trifoliate leaves. Leaflets are elliptic and acute with hairy forms whereas the fruits are thick. Inside fruits, seeds are arranged in compressed form with square type seeds. The plant is native to India and well distributed in Rajasthan, Haryana, Gujarat and Punjab (**Bagenia and Chaturvedi, 2018**). The plant is traditionally used for arthritis, enlarged liver, head-swellings, laxative and in asthma (**Sharma *et al.*, 2010**). The plant is also having multiple therapeutic activities viz. antidiabetic (**Saeed *et al.*, 2012**), antiviral (**Kaushik *et al.*, 2020**), anticoagulant activity (**Hassan *et al.*, 2010**), antimicrobial (**Jerine Peter *et al.*, 2020**), antiasthamatic (**Sharma *et al.*, 2010**), Anthelmintic (**Tahmouzi *et al.*, 2023**) and wound healing (**Tahmouzi *et al.*, 2023**) activities etc. All these activities are mainly due to presence of a vast number of active constitutions. It was reported that bioactive compounds as a source of secondary metabolites in plants, are responsible for various activities (**Seca and Pinto, 2018**). Very scanty reports on antioxidant study on the said plant (**Seema *et al.*, 2011**). Furthermore, metallic ions are very essential for production of secondary metabolites. There are many literatures available on the same (**Locatelli *et al.*,2016; Das *et al.*,2019**) but no such literature available on the said plant.

Furthermore, *Ficus elastic* Roxb. ex Hornem(FE) (F: Moraceae) is commonly known as rubber plant, having large green color leaves (around 10 to 35 cm in length) with pink stipules. The plant is widely distributed in India, Nepal, Buthan, Myanmar, Malaysia, and Indonesia (**Arsyad *et al.*, 2023**). Traditionally, the plant is used in digestive, reproductive, endocrine, respiratory systems, gastrointestinal, cardiovascular diseases, diabetes, stomach ulcer, muscle pain and recently it is used in cancer therapy (**Shi *et al.*, 2018; Gurung *et al.*, 2021**).It has many pharmacological activities with the presence of various phytochemicals in various parts of the plants viz. antibacterial, antifungal, and antiproliferative properties due to the active principles of Ficusamide, ficusoside B, elastiquinone, elasticoside, and elasticamide.Very scanty reports are available on antioxidant property of the said plant (**Phan Van *et al.*, 2012; Mbosso Teinkela *et al.*, 2016**).

Many literatures already reported antioxidant activity of many plant extracts with various *in vitro* assay methods, but no literature told the role of metal ion content in the plant and their role in antioxidant activity. Thereafter, an interrelationship among the identified plant constituents along with the metal ion content and antioxidant activity was carried out in the present investigation which was first time report on the selected plant duos.

MATERIALS AND METHODS

Chemicals 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and Quercetin was purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA). Sodium carbonate (Na_2CO_3), Sodium phosphate (NaH_2PO_4) was purchased from Himedia Laboratories Pvt. Ltd. (Mumbai, India). Methanol, Ferric chloride (FeCl3), Potassium Ferricyanide ($K_3Fe(CN)_6$), Trichloroacetic acid, Folin- Ciocalteau reagent, Methanol, Ascorbic acid, Gallic acid was purchased from SRL Pvt. Ltd. (Mumbai, India). Ammonium molybdate ($(NH_4)_2MoO_4$) and Aluminium chloride ($AlCl_3$) were purchased from SD Fine-Chem Chem. Ltd. (Mumbai, India). All other chemicals used were of analytical grade.

Collection of Plant Materials

The plant materials CT and FE were collected from cultivated zone of Palakard, Kerala and botanically authenticated by Dr. K. Madhava Chetty, Sri Venkateshwara University, Tirupati, Andhra Pradesh, India. A voucher specimen with accession number 0597 and 0908 respectively was deposited to the said plant material.

Preparation of plant sample for metal ion determination

Using an Atomic Absorption Spectrophotometer (AAS, Perkin Elmer, PinAAcle 900T, Mumbai, India), 100 g of dried powder was utilized to determine the elements, particularly a few heavy metals and several important elements contained in the leaves. Three potent acid combinations were used to break down the powdered leaves. Concentrated nitric acid, concentrated sulfuric acid, and 60% perchloric acid were combined to form a triacid mixture (100: 10: 40). Fe, Mn, Mg, Cu, Zn, Pb, Cd, Ni, As, and Cr were estimated. 15 ml of ternary acid combination, which had been earlier made with three concentrated acids, was combined with 5 g of powdered plant samples. The mixture was then digested at 180 to 200 degrees Celsius until dense white vapors evolved and produced residue. The residue was filled with a volumetric flask to a specific capacity after being further diluted with glass distilled water. After that, the mixture was prepared for the analysis of harmful heavy metals such as Cd, Cr, Pb, As, and Ni, as well as Fe, Cu, Mn, Mg, and Zn with slit 0.7 (Atomic Absorption Condition) and 0.2 (Flame Emission Condition) by using air acetylene gas. Further some macronutrients like Na, and K were determined by flame photometry method whereas Mg, and Ca, were analyszed by Atomic Absorption spectrophotometer (**Ahmed, 2018; Das *et al*., 2019**).

Preparation of Plant extracts

500 g of shade dried leaves (dried for 28 days) of *Ficus elastica* and beans of *Cyamopsis tetragonoloba*, were subjected to course powdered by mixer grinder and further extraction using ethanol as solvent by soxhlet method. The extraction condition was 45 degree C for 8 hrs followed by filtration of the extracts and evaporation of the excess solvent using rotary flash evaporator (Remi, Bio Techno Lab, Mumbai, India) at 40 degree C for 45 min. Resulted viscous extract was weighed for practical yield and preserved in screw capped glass bottle by kept in refrigeration condition at 4 degree C for further investigation.

Phytochemical screening

Various chemical tests were conducted with the extracts as per the method described earlier. The tests were series of qualitative chemical tests targeting specific classes of compounds, such as alkaloids, flavonoids, glycosides, tannins, saponins, and other phytochemicals.

TLC and HPLC analysis

Further, the extract was run with TLC for identification and separation of the phytoconstituents. The mobile phase was used as benzene and methanol (9:1) for identification of *beta* sitosterol and lupeol.
Standard Lupeol and β-sitosterol were procured from Sigma-Aldrich (Bengaluru, India)(Purity of standard leupeol was 99.5% and *beta* sitosterol was 98.5%), and was used as standard for the HPLC analysis (Shimadzu, Mumbai, India). C18 column was used and methanol, water (70: 30) was used as mobile phase. Detection was carried out at 208 nm after prepared sample and standard solutions. Injection was 10 µl with flow rate 1.0 ml/min.

Determination of total Phenolics

Modified Folin–Ciocalteu colorimetric method was applied for analysis of total phenolic content for both the said ethanol extracts using 650 nm as absorbance (**Siddiqui *et al*., 2017**). Gallic acid in varied concentration was prepared and series of absorbance recorded. Finally, absorbance vs concentrations were plotted as standard curve.
Sample (1mg/ml) was mixed in 1ml of Folin-phenol Ciocalteu's reagent separately and then total phenolic content was measured in mg gallic acid equivalents (GAE) per gram of dry weight (mg/g).

Antioxidant Activity

DPPH free radical scavenging activity

The DPPH Assay was conducted as per the method followed earlier with slight modifications (**Valko *et al*., 2007**). 1000 microliters of different concentrations (5-30 µl/ml) of the extracts dissolved in ethanol were added to 4 ml of a 0.004% methanol solution of DPPH. Following a 30-minute incubation period at room temperature, the absorbance was measured at 517 nm against a blank and IC50 was calculated from % inhibition. Ascorbic acid was used as a standard and dissolved in distilled water to make the stock solution with the same concentration of ethanol extracts of FE and CT plants. The DPPH radical scavenging effect was calculated as the percentage inhibition (I%) using the formula:
% inhibition=$(1-A_{blank}/A_{Sample})\times100$
Where A_{blank} is the absorbance of the control reaction containing all reagents except the test compound, and A_{sample} is the absorbance of the test compound. The inhibition values were calculated for various concentrations of the extracts from FE and CT. The experiments were performed in triplicate to ensure accuracy and reproducibility of results.

Hydrogen Peroxide Scavenging Activity

Initially, a solution of hydrogen peroxide (H_2O_2) at a concentration of 40 mM was prepared in phosphate buffer (pH 7.4). Subsequently, extracts at concentrations ranging from 10 to 320 µg/ml dissolved in methanol were added to 0.6 ml of the H_2O_2 solution. The absorbance of the reaction mixture was then measured at 230 nm (Hazra *et al*., 2008). A blank solution, consisting of phosphate buffer without H_2O_2, was included for reference. The percentage of hydrogen peroxide scavenging activity was calculated using the following formula:
% Inhibition=$(A_{control}-A_{sample}/A_{control})\times100$
Where $A_{Control}$ represents the absorbance of the control (i.e., the reaction mixture without the sample or standards), and A_{sample} is the absorbance in the presence of the sample or standards. This assay provides insights into the ability of the extracts to scavenge hydrogen peroxide, an important reactive oxygen species implicated in oxidative stress-related diseases.

Correlation study

Metal ion content was correlated with the total phenolic content and antioxidant activity and further total phenol content was correlated with the potent antioxidant activity and results were compared among the two said plants.

Statistical interpretation

All the values were replicated thrice, mean ± SEm (Standard Error Mean) (n=3). Thereafter, the significant difference at $p < 0.05$ was determined and the means were statistically analyzed using a one-way ANOVA.

RESULTS

Analysis of Elements

All the said elements were analyzed by Atomic Absorption Spectrophotometer and resulted Zn and Cu content were higher in leaves of FE plant whereas, Mg and Cu content were higher in CT beans. Thereafter, content of Fe and Mn content was lesser than Zn and Cu in both the plants (Table-1). Interestingly, non- essential heavy metals viz. Ni, Cd, As, Pb and Cr content were negligible to below detectable level that indicated risk factor was less. Higher amount of Na, Ca and K were resulted from both the plants.

Table1 Analysis of Elements in dried plant samples

Elements	Slit	Gas used	FE plant (mg/kg)	CT plant (mg/kg)
Zn	0.7 (Atomic Absorption Condition) 0.2 (Flame Emission Condition)	Air Acetylene	5.23 ± 0.23	3.46 ± 0.27
Cu		Air Acetylene	4.29 ± 0.11	4.08 ± 0.53
Fe		Air Acetylene	2.10 ± 1.04	1.37 ± 1.20
Mg		Air Acetylene	1.21 ± 0.06	3.82 ± 0.41
Mn		Air Acetylene	0.97 ± 0.25	0.84 ± 0.23
As		Air Acetylene	ND	ND
Co		Air Acetylene	0.012 ± 1.27	0.010 ± 1.33
Ni		Air Acetylene	ND	ND
Pb		Air Acetylene	ND	ND
Cr		Air Acetylene	ND	0.003 ± 0.01
Na			31.8 ± 0.37	39.8 ± 0.67
Ca			4.9 ± 0.21	11.6 ± 1.21
K			26.7 ± 0.20	34.4 ± 1.04

Mean ± SEm (n = 3); ND = Not detected

Extraction of plant materials

500 g of dried powdered samples were extracted by ethanol and resulted the practical yields were 67.7 g and 84.3g for FE and CT plants respectively. The percentage yield was tabulated in figure-1.

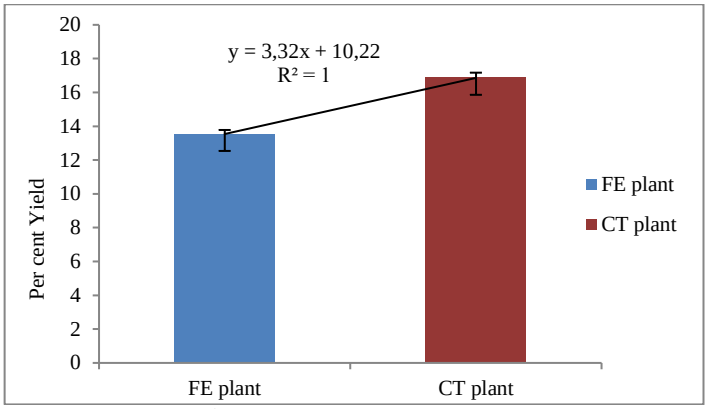

Figure1 Percent yield of both the FE and CT plant ethanol extract

Phytochemical Screening of Extracts

In the present study, various chemical tests were performed and resulted the presence of carbohydrates, proteins, tannins, alkaloids, flavonoids, glycosides, steroids, terpenoids, and amino acids in FE extract whereas carbohydrates, flavonoids, glycosides, terpenoids, tannins, steroids, and amino acid were present in CT extract (Table-2).

Table2 Qualitative analysis of Phytochemicals of FE and CT extracts

Name of chemical test	FE extract	CT extract
Alkaloids	++	++
Protein	+	++
Carbohydrate	+	+
Glycosides	+	++
Tannins and Phenolic compounds	++ +	+++
Flavonoids	++	+++
Steroids	++	+++
Amino acids	+	+
Saponins	+	++
Terpenoids	++	+++

(+++ indicates strongly present, ++ indicates moderately present,+ indicates less presence)

TLC and HPLC analysis

Based on the chemical tests, further, TLC was performed and resulted presence of steroidal compound and terpenoids when compared with the standard phytochemicals. It was observed that, *beta* sitosterol was identified in both the extracts with the Rf values of 0.57 and 0.59 for FE and CT respectively (Figure-2) and lupeol was identified at Rf of 0.36 and 0.34 for CT and FE extract respectively.

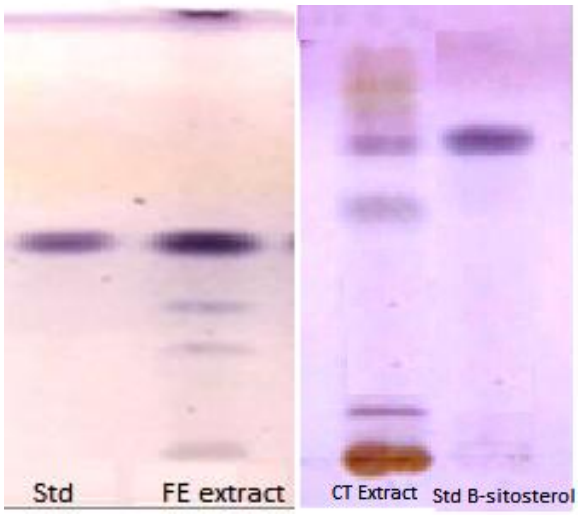

Figure 2 TLC of FE and CT extract for presence of *Beta*-Sitosterol

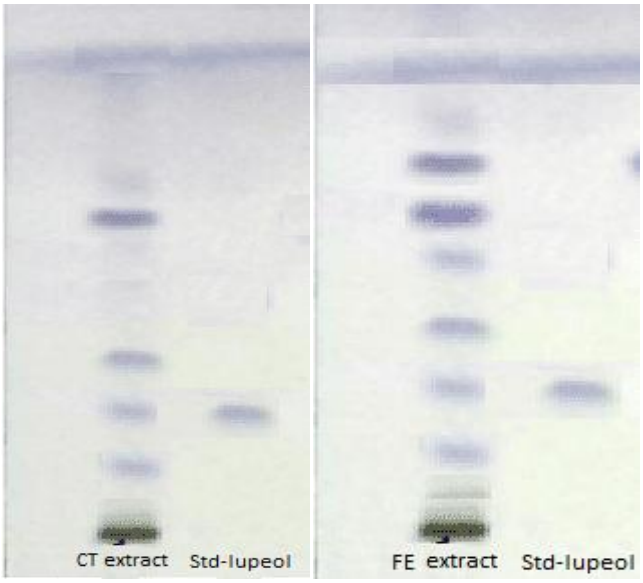

Figure 3 TLC of FE and CT extract for presence of Lupeol

Based on the TLC, further HPLC was carried out and observed that at Rt of 5.15 min lupeol was eluted followed by *beta* sitosterol was eluted at Rt of 8.89 min in the CT and FE extracts respectively (Figure-4 and 5). The same were confirmed with standard lupeol and *beta* sitosterol graphs (Figure-6 and 7).

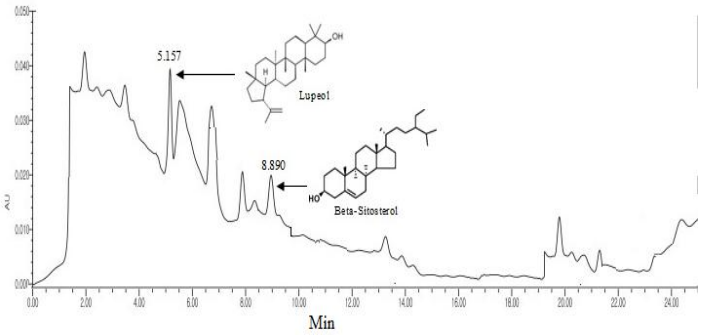

Figure 4 HPLC graph for CT beans extract

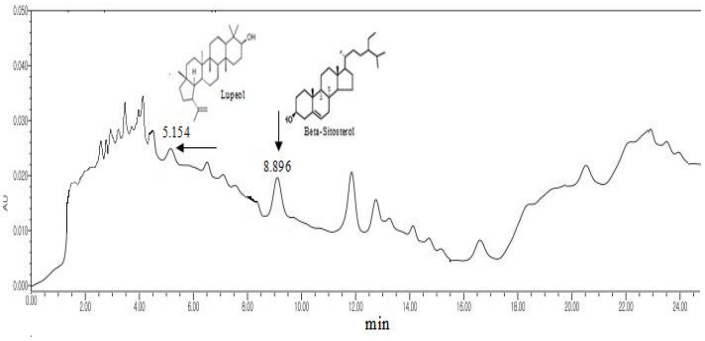

Figure5 HPLC graph for FE leaves extract

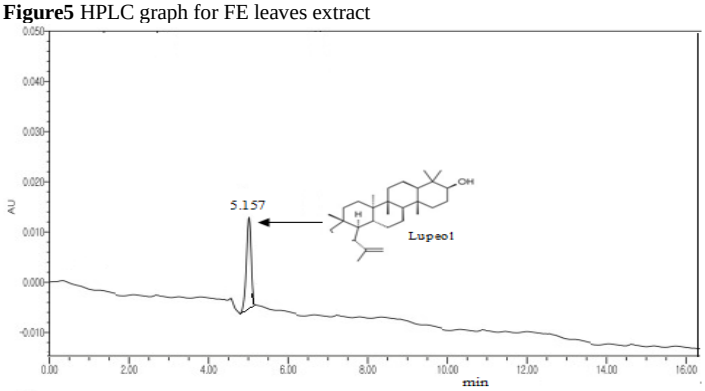

Figure6 HPLC graph for standard Lupeol

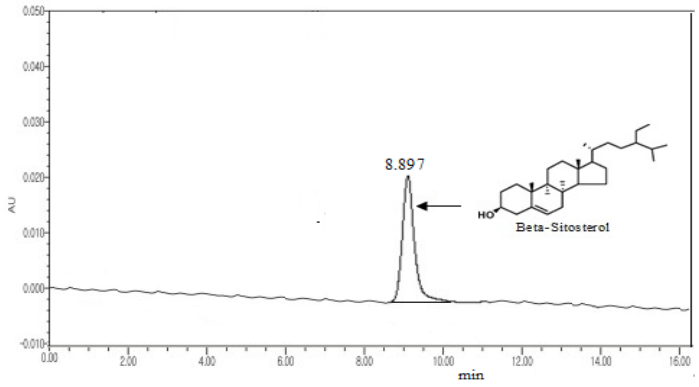

Figure 7 HPLC graph for standard *beta*-sitosterol

Content of constituents was listed in table-3, where *beta* sitosterol content was more in FE extract and Lupeol content was more in CT extract.

Table 3 Quantitative estimation of CT and FE extracts

Extracts	*Beta* sitosterol (mg)	Lupeol (mg)
FE extract	6.48 ± 0.23	3.01 ± 0.41
CT extract	3.41 ± 0.11	5.37 ± 0.26

Values are represented as mean ± SEm (n=3)

Total phenol content

The total phenol content in FE and CT was determined using the FC (Folin-Ciocalteu) reagent method. A standard curve of gallic acid (standard phenol) was constructed to quantify the total phenol content. The linear equation obtained from the standard curve was($y = 0.0007x + 0.067$; $R^2 = 0.999$) In this equation, y represents the absorbance measured after the reaction of extracts with the FC reagent at 650 nm, and x represents the concentration of gallic acid per milliliter(Figure-8).

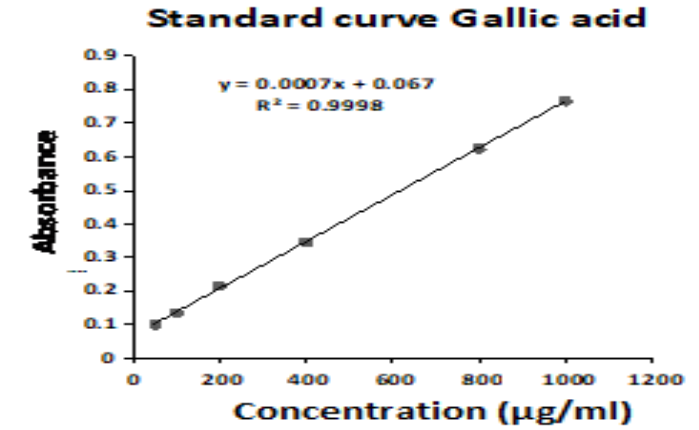

Figure 8 Standard gallic acid curve

The absorbance of FE sample was 0.217 and CT sample was 0.326.
Now, based on the standard curve of Gallic acid, absorbance of the samples was replaced in "y" value and from the above results, the total phenol content in CT and FE was calculated to be 370 and 214.28 milligrams of gallic acid equivalent per gram (where the absorbance of CT was 0.326 and FE was 0.217).

Test for Anti-Oxidant Activity

DPPH scavenging assay

In this assay, the scavenging potential of FE and CT was observed to be concentration-dependent, with the scavenging activity increasing as the concentration of the extracts increased. The results are presented as percent inhibition (mean) ± SEm and IC50 (half-maximal inhibitory concentration) values in micrograms per milliliter (µg/ml). Standard ascorbic acid graph was plotted (5 to 30 µg/ml) and from that IC50 value was determined after calculation of percent inhibition (Figure-9).
At a concentration of 50 µg/ml, the DPPH scavenging activity of CTwas determined as84.75 ± 0.06%, while for FE, it was 79.53 ± 0.14%. Additionally, the scavenging potential of both the plant extracts was compared with that of the standard antioxidant, ascorbic acid. The DPPH scavenging activity of ascorbic acid was found to be 98.08 ± 0.22 %. Thereafter, the IC50 values for all was calculated from the standard graph where ascorbic acid was determined as 3.09 µg/ml (Figure-10), while those of FE and CT were found to be 3.78 µg/ml and 3.45 µg/ml, respectively (Table-4).

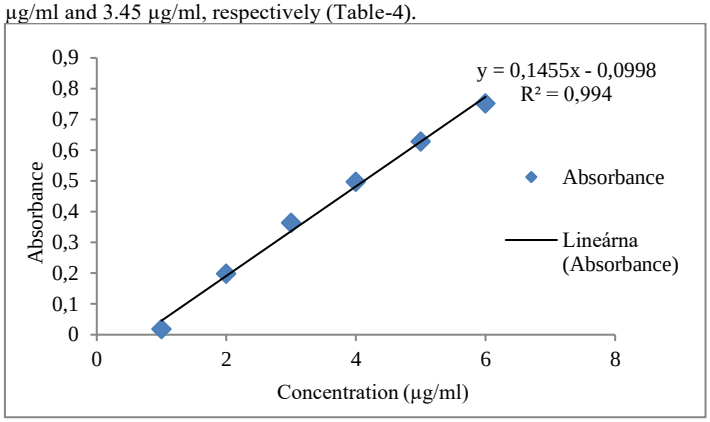

Figure 9 Standard curve for Ascorbic acid

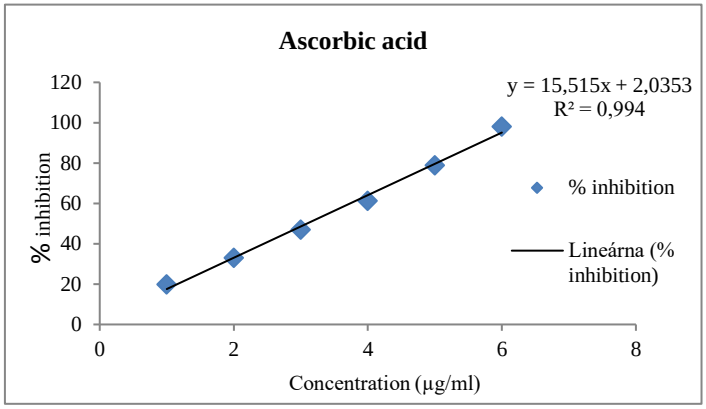

Figure 10 Per cent inhibition of Ascorbic acid

Table 4 Per cent inhibition of CT, FE and ascorbic acid with IC50 value in DPPH method

Component	Conc. (µg/ml)	% inhibition	IC50 value (µg/ml)
Ascorbic acid	5	19.82 ±0.23	3.09
	10	33.04 ±0.11	
	15	47.01 ±0.26	
	20	61.19 ±0.36	
	25	78.89 ±0.42	
	30	98.08 ±0.31	
CT extract	05	13.64±0.30	3.45
	10	25.47 ±0.23	
	15	44.45 ±0.11	
	20	58.74 ±0.72	
	25	77.50 ±0.32	
	30	84.75 ±0.06	
FE extract	05	9.38 ±0.01	3.78
	10	23.13 ±0.31	
	15	38.59 ±0.22	
	20	56.28 ±0.11	
	25	69.08 ±0.41	
	30	79.53 ±0.14	

Values are mean ± SEm; (n =3).

Hydrogen peroxide scavenging assay

The similar effect resulted with the hydrogen peroxide assay method where dose dependent activity was recorded. The antioxidant activity of FE against hydrogen peroxide radical was determined as62.73 ± 0.62% at 320 µg/ml while for CT, it was found to be 63.58 ± 0.28% at the same concentration (Figure-11). Comparatively, the percent inhibition of hydrogen peroxide by ascorbic acid was found to be 67.23 ± 0.56%. Furthermore, the IC50 values, indicating the concentration required to inhibit hydrogen peroxide radical activity by 50%, were determined. The IC50 value of ascorbic acid was calculated to be 99.55 µg/ml. In contrast, the IC50 values of CT and FE were found to be 134.78 µg/ml and 146.66 µg/ml, respectively (Table-5).

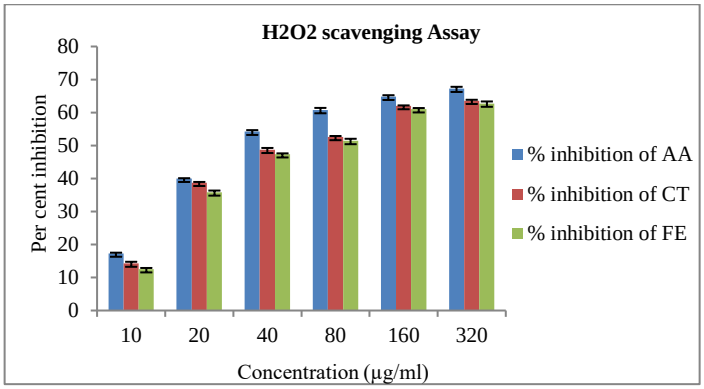

Figure 11 Comparative hydrogen peroxide scavenging activity between Ascorbic acid (AA) and extracts

Table 5 Per cent inhibition of CT, FE and ascorbic acid with IC50 value in H_2O_2 scavenging assay

Component	Conc. (µg/ml)	% inhibition	IC50 value (µg/ml)
Ascorbic acid	10	17.29 ±0.23	99.55
	20	39.95 ±0.14	
	40	54.20 ±0.46	
	80	60.77 ±0.62	
	160	64.79 ±0.45	
	320	67.23 ±0.56	
CT extract	10	14.25±0.52	134.78
	20	38.73 ±0.23	
	40	48.72 ±0.54	
	80	52.61 ±0.27	
	160	61.99 ±0.16	
	320	63.58 ±0.28	
FE extract	10	12.55 ±0.34	146.66
	20	35.81 ±0.54	
	40	47.38 ±0.24	
	80	51.40 ±0.64	
	160	61.02 ±0.33	
	320	62.73 ±0.62	

Values are mean ± SEm; (n =3).

Correlation study

Metal ion content was correlated with the presence of plant secondary metabolites and helped in content of *beta* sitosterol and lupeol content and showed positive correlation (Table-6 and 7).

Table 6 Correlation study between Zn content in extracts with secondary metabolites

	Zn in FE extract	*Beta*-sitosterol in FE	Lupeol in FE extract
Zn in FE extract	1		
Beta-sitosterol in FE	0.998**	1	
Lupeol in FE extract	0.979*	0.962	1

Significant at *p<0.05; **p< 0.01

Table 7 Correlation study between Cu content in extracts with secondary metabolites

	Cu in CT extract	*Beta*-sitosterol in CT	Lupeol in CT extract
Cu in CT extract	1		
Beta-sitosterol in CT	0.978**	1	
Lupeol in CT extract	0.943*	0.577	1

Significant at *p<0.05; **p< 0.01

Similarly, total yield of the extract also showed positive correlation with the separated compounds (Figure-12). Thereafter, yield of FE extract also showed positive correlation with the content of *beta* sitosterol (Figure-13).

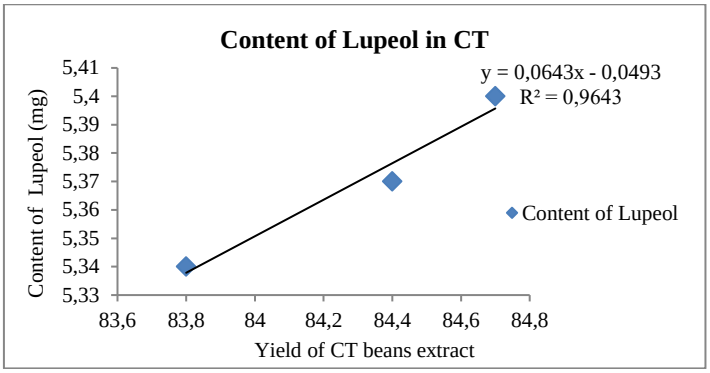

Figure 12 Correlation study of Lupeol content with yield of CT extract

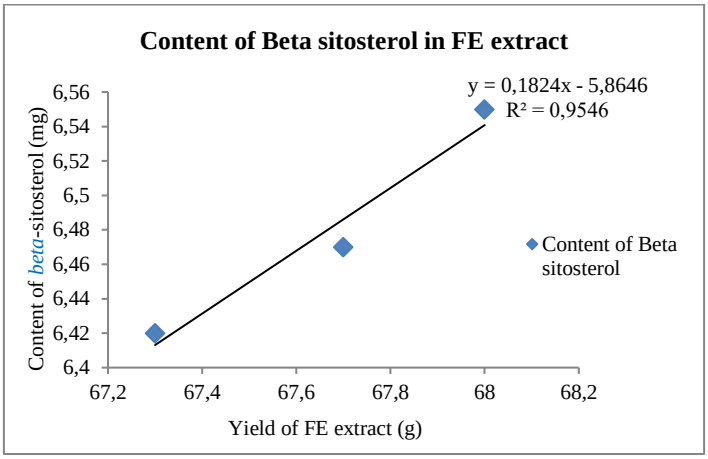

Figure 13 Correlation study of *Beta*-sitosterol content with yield of FE extract

Furthermore, yield of the extracts were correlated with the total phenolic content (Table-8) and total phenolic content was further correlated with the antioxidant study (Figure-14 and 15). Interestingly, in both the cases showed positive correlation.

Table 8 Correlation study among the extracts with total phenolics content

	Yield of FE	Yield of CT	Total phenolics in FE extract	Total Phenolics in CT extract
Yield of FE	1			
Yield of CT	0.996**	1		
Total phenolics in FE extract	0.950*	0.877	1	
Total Phenolics in CT extract	0.954	0.929	0.572	1

Significant at *p<0.05; **p< 0.01

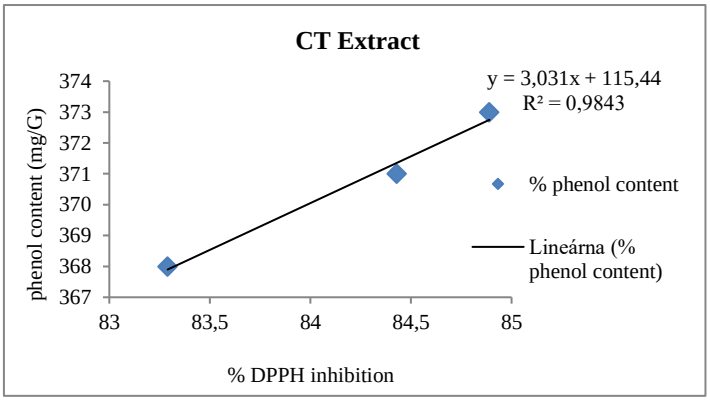

Figure14 Correlation study of total phenolics with DPPH inhibition activity

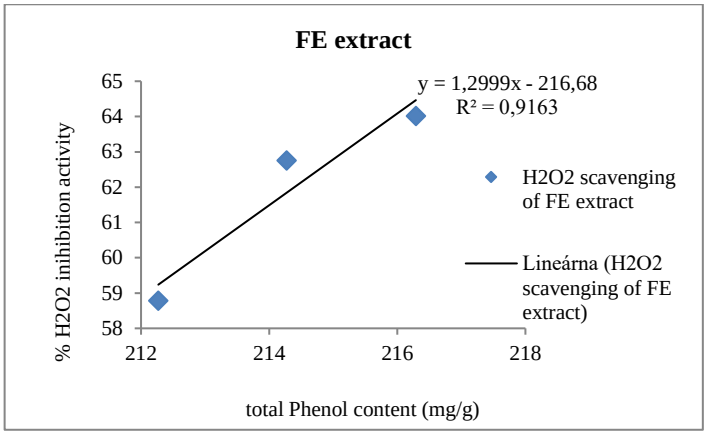

Figure 15 Correlation study of total phenolics with H_2O_2 scavenging activity

DISCUSSION

Macro and micro elements plays a major role in biosynthesis of many secondary metabolites and actively participate in the growth of the plants. Based on the concept, in the present study elemental analysis for the dried leaves and beans samples of FE and CT plants was carried out where Zn and Cu content were higher in FE leaves which were essential for production of many secondary metabolites. Thereafter, Mg and Cu content were higher in CT dried beans. Hence, the content of secondary metabolites enhanced. Earlier literatures also revealed the same (**Das and Tribedi, 2015**). As per WHO guideline, optimum Cu content recorded as 5 to 30 mg/kg and the optimum Mg concentration ranges 0.09–0.40% for woody plants are satisfactory for the plant growth. Zn content 30 and 100 µg/g dry weight is optimum for plant growth (**Das *et al*., 2017**). Thereafter, ethanol solvent was used for the extraction which was safe and easily available. Ethanol was the best solvent because it has high dielectric constant and easily available. Thereafter, maximum bioactive compounds are highly soluble in ethanol and hence ethanol was the best choice of solvent (**Das *et al*., 2023; Das and Singirikoda, 2023**). Earlier literature also revealed the same with the ethanol solvent which enhanced the solubility of maximum plant secondary metabolites from plants (**Mahardika and Roanisca, 2019; Huaman-Castilla *et al*., 2019**). Due to ethanol solvent, the present study showed higher yield in CT beans extract than FE leaves extract.

Further, various phytochemical studies for both the extracts were carried out and revealed the presence of many groups of plant constituents and based on that further, TLC was performed to detect and separate the specific constituents. It was showed that presence of lupeol and *beta* sitosterol as major constituents in the both plants commonly. Many literatures revealed the TLC of lupeol and *beta* sitosterol in various plants extracts (**Thatipelli *et al*., 2023**). It was revealed that higher content of *beta* sitosterol and lupeol with the higher yield of the plant extracts. Total phenolics was determined and showed positive correlation with the yield. Earlier literature also reported that the total phenolic content dependent on solvent used for extraction and also on the yield of the extract (**Noreen *et al*., 2017**).

Finally, antioxidant activity was carried out by DPPH and hydrogen peroxide scavenging assay methods. Free radicals are thought to have a significant role in the onset of chronic illnesses. Because of their demonstrated antioxidant activity and ability to reduce oxidative stress-induced tissue damage, which is linked to a number of chronic diseases, plant polyphenols are crucial parts of the human diet (**Rio *et al*., 2013**). Since the majority of phenolic chemicals are polar, higher polarity solvents like ethanol have been used to effectively and yield-fully extract them. Across the range of extracts examined, a significant difference in total phenolic content was noted, with ethanol extract exhibiting the largest variation, up to a 15-fold increase in the present study.The current research, which is consistent with earlier findings, demonstrated a favorable correlation between high antioxidant activity and phenolic content for several extracts of this plant (**Jorge *et al*., 2015**).Many other literatures also revealed correlation study between the yield with total phenolic content, antioxidant properties and correlation among metal ions (**Das *et al*., 2019; Das *et al*., 2022**). Further it was observed that IC50 value was inversely proportional with the antioxidant activity but the antioxidant activity was showed as dose dependent. Many literatures also reported that lesser IC50 values indicate higher the activity. In the present study, the same result observed (**Rivero-Cruz *et al*., 2020; Itam *et al*., 2021**). Though both the said plant extracts showed positive antioxidant activity but interestingly, CT beans extract showed higher result in terms of yield, total phenolic content, an even antioxidant activity than FE leaves extract due to presence of major macronutrients in high content than FE leaves. The result was similar with the earlier report (**Rana *et al*., 2019**).

CONCLUSION

In the present study, *beta* sitosterol and lupeol was identified and estimated from FE and CT plant ethanol extracts. Further, the study revealed the presence of antioxidant property and step by step correlated with the element content in plants; their role in production of secondary metabolites, total phenolics content and their effects on antioxidant property was established. This was first time established the correlation study among the elemental content, yield, phenolics content and antioxidant property. Dose dependent antioxidant activity with the presence of phenolic compound and triterpenoids was established and among them CT extract showed comparatively higher than FE extract but both are potential source in pharmaceutical products to enhance human health by strengthening the antioxidant defense system's ability to fend off the generation of free radicals. In future, they will help in food preservation, sources of potent antioxidant and protect products from microbial attack.

Further, it is necessary to isolate the compounds responsible for activities and molecular study for the establishment of mechanism for therapeutic activities.

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