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Research Article

Phytochemical Characterization, Anti-oxidant and Anti-diabetic Activities of Unexplored Endemic Species *Cotoneaster buxifolius* Leaves

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ABSTRACT

There is a growing interest in natural products for their combined nutritional and medicinal benefits. Plants are widely studied as sources of nutraceuticals that exhibit notable therapeutic effects. Cotoneaster buxifolius is a medicinal plant which is traditionally used by the Thoriya ethnic group to treat fungal infections. Despite its ethnobotanical use, no prior scientific evaluation of its medicinal properties has been reported. The present study involved phytochemical screening, quantification of primary and secondary metabolites, antioxidant (DPPH, FRAP, Phosphomolybdenum and Superoxide assays), anti-diabetic activity and FT-IR analysis of C. buxifolius leaves. The leaf extract showed significant levels of carbohydrates (70.3 mg GE/100 g), protein (40.30 mg BSAE/100 g), amino acids (16.24 mg LE/100 g), phenols (981.99 mg GAE/g), flavonoids (319.76 mg RE/g) and tannins (272.8 mg GAE/g). The ethanol extract demonstrated strong antioxidant and anti-diabetic activities and FT-IR analysis identified twelve absorbance peaks. These findings support the plant's potential for future pharmacological applications, especially in natural antioxidant and anti-diabetic therapies.

INTRODUCTION

Traditional herbal medicine has a long history across cultures, with knowledge passed down through generations [1]. While modern medicine has become widespread, it is often hindered by significant side effects and limited affordability. There is a pressing need to explore natural medicines that offer comparable therapeutic benefits with fewer side effects or no side effect. Herbal remedies are more accessible and cost-effective than conventional pharmaceuticals in certain regions, particularly where access to modern healthcare is constrained [2]. Phytochemicals are bioactive compounds found in plants that offer a diverse array of therapeutic

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benefits. Recent research has brought renewed attention to the medicinal value of herbal plants and their potential to address global health challenges [3].

The Rosaceae family is a diverse group that encompasses a broad spectrum of economically important plants, including nuts, aromatic species, fruits and ornamentals. The *Cotoneaster* genus which belongs to the Rosaceae family, encompasses over 500 species distributed throughout the Himalayan and Chinese mountain regions [1].

The *C. nummularia* ripened fruits are utilized as an expectorant and antipyretic, providing relief for malarial fever, while the roots are used to alleviate rheumatic conditions. The leaves of *C. accuminata* are applied externally to ease rheumatoid arthritis and are used to support the treatment of scabies and its roots are effective in lowering blood pressure. A decoction made from the *C. integerrimus* leaves and fruits is recommended for its strong antimicrobial properties, aiding in the diabetes, cough, therapy for digestive ailments and fever. Various *Cotoneaster* species have been widely utilized in ethno medicine to resolve various conditions, such as nasal hemorrhage, menorrhagia, hemorrhoids, cardiovascular disorders, diabetes, neonatal jaundice, cough, as a laxative, astringent, hepatoprotective agent, digestive aid, antiviral agent and spasmolytic properties, as well as to treat eye and bronchi infections, strangury, thirst, itching, fever, lesions and urinary calculi [1].

Phytochemical analysis of *Cotoneaster* species has revealed a diverse array of unique compounds including phytoalexins, proanthocyanidins, flavonoids, phenolic acids, triterpenoids and cyanogenic glycosides in different plant parts. Despite these traditional medicinal uses, the genus *Cotoneaster* remains largely understudied and its phytochemical and pharmacological potential remains underexplored [1].

Cotoneaster buxifolius Wall. Ex Lindl. is an endemic plant native to the Sholur region of the Nilgiris district in Tamil Nadu, India. This shrub-like plant commonly called as Kallu mora by Badaga tribal people and Box-Leaves in English has been traditionally used in indigenous medicine. The Thoriya ethnic (Sub-tribe of badagas) people have utilized the petals and leaves to treat fungal infections both externally and internally [4].

A comprehensive review of the existing literature has revealed that no prior studies on investigating the anti-oxidant and anti-diabetic characteristics of *C. buxifolius* leaves are published Therefore, the current investigation aims to determine the phytochemical profile, quantify the primary and secondary metabolites and evaluate the antioxidants,
anti-diabetic activity and FT-IR analysis of various extracts derived from *C. buxifolius* leaves to identify the functional groups.

MATERIALS AND METHODS

Collection, Preparation and Extraction of Plant Materials

The leaves of *Cotoneaster buxifolius* were collected during the months of June and July from Sholur, The Nilgiris district, Tamilnadu, India was identified by its taxonomic identity comparing the voucher specimen at the Madras Herbarium (MH) of Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu (Ref.no. BSI/SRC/5/23/2022/Tech/217).

Freshly collected leaves were cleaned and shade dried for 3 weeks. The desiccated samples was coarsely powdered for further investigation. The

powdered leaf samples of *C. buxifolius* was extracted successively by cold & hot maceration using successive extraction method in the increasing order of the polarity with different solvents such as petroleum ether, chloroform, ethyl acetate, ethanol and water. The oven dried extracts were weighed and stored on low temperature (-20°C). The extract thus obtained was used for various analysis.

The extract yield percentage were calculated using following formula,

$$\text{Extract recovery Percentage} = \frac{\text{Amount of extract recovered (g) X100}}{\text{Amount of leaf sample (g)}}$$

Qualitative Phytochemical Screening

Qualitative examination of phytochemical screening of leaf powder was carried out to determine the major groups of compounds present in the extracts according to standard procedure of Raaman (2006)[5].

Quantitative Analysis of Total Carbohydrate, Protein and Amino acid

The total carbohydrates, protein and amino acid contents of the powdered plant sample were quantified by following the method Sadasivam and Manickam[6]. For calibration, glucose, bovine serum albumin (BSA), leucine was used as standards for carbohydrate, protein and amino acid respectively.

Quantification of Total Phenolic and Tannin content [7]

For total phenolics estimation aliquots (0.2–1.0 ml) of a gallic acid standard solution was prepared in test tubes (S1–S5). 500 µl of extract was added to separate tubes and all analyses were performed in triplicates. The volume was adjusted to 1 ml with distilled water and 0.5 ml of 1N Folin-Ciocalteu reagent was added. After 5 min, 2.5 ml of 5% sodium carbonate was mixed, followed by incubation in the dark for 40 min. Absorbance was measured at 725 nm and total phenolics were expressed as mg gallic acid equivalents (GAE)/g sample.

For tannin estimation, 100 mg polyvinyl polypyrrolidone (PVPP) was mixed with 500 µl of the sample and 500 µl of distilled water, incubated at 4°C for 4 h and centrifuged at 3000 rpm for 10 min. The supernatant (non-tannin phenolics) was analysed similarly to total phenolics, using a gallic acid standard curve. The tannin content was calculated as:

$$\text{Tannins (g)} = \text{Total phenolics (g)} - \text{Non-tannin phenolics (g)}$$

Quantification of Total Flavonoid Content [8]

About 500 µl of extracts in triplicates were taken and it was made up into 1ml with distilled water. Distilled water was served as blank. Then, 150µl of 5% sodium nitrite was added to each samples and subsequently incubated for 6 min in room temperature. After incubation, 150 µl of 10% aluminium chloride was added to the samples including the blank. The reaction mixture was incubated for 6 min at room temperature. 2 ml of 4% NaOH was added and the total volume was made up to 5 ml with distilled water. The contents were vortexed and incubated for 15 min at room temperature. Flavonoid content was measured at 510 nm and the results were expressed in Rutin equivalents (RE) per gram sample.

Antioxidant Assays

DPPH + (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay [9]

The DPPH+ was used to measure the free radical scavenging activity of leaf extracts of *C. buxifolius*. Various concentration of leaf extracts and standards (rutin) were obtained and 3 mL of 0.1 mM methanol solution of DPPH was added. For negative control 100 µL of methanol and 5 mL of DPPH solution was used. All the reaction mixtures were incubated in dark at 27°C for 20 min. Inhibition of DPPH radicals by the plant extracts was measured at 517 nm against the blank (methanol).

Phosphomolybdenum assay

Total antioxidant capacity of the extracts was measured by phosphomolybdenum reduction assay depicted by Prieto et al. (1999) [10]. The 1mL of reagent solution was added to the extracts and standard. Further, the test tubes were incubated for 90 min at 95°C. At 765 nm the absorbance of the mixture was recorded against the blank. The results were reported in mean values and expressed as ascorbic acid equivalents (AAE) per gram sample.

Ferric reducing antioxidant power (FRAP) assay

Determination of ferric reducing ability in the extracts were characterized by the method Pulido et al. (2000)[11]. The aliquots of sample and standard were taken in test tubes and then added 900 µL of FRAP reagent was added. Then the test tubes were incubated for 30 min at 37°C. After incubation, intensity was measured at 593 nm. The findings were expressed as Fe (II) equivalents per gram of substance.

Superoxide anion radical scavenging assay [12]

The assay was carried out to evaluate the ability of different extracts in inhibiting formazan production by scavenging superoxide radicals generated in riboflavin – light- NBT system (Beauchamp and Fridovich, 1971). Standard and samples were taken in the test tubes and 3 mL of reaction mixture was added. Each 3 mL reaction mixture carry a 50 mM sodium phosphate buffer (pH-7.6), 20 µg riboflavin, 12 mM EDTA, 0.1 mg NBT and 40 µL of aliquot of sample solution or rutin (standard). The photo-illumination was induced by 20W fluorescent lamps. The reactant samples were illuminated at 25°C for 10 min. The un-illuminated reaction mixture was used as a blank and measured against 590 nm. The rutin was used as a standard. The results were expressed as percentage of superoxide anion radical inhibition. Test tubes with reaction mixture kept in the dark served as negative control.

The scavenging activity on superoxide anion generation was calculated as:

Scavenging activity (%) = [(A0 - A1) / A0] X 100

Where,
A0 is the absorbance of the control and
A1 is the absorbance of the sample extract/standard.

Determination of *In vitro* Anti-diabetic Activity

α -amylase inhibition activity

The inhibitory effect of the extracts on α-amylase was evaluated using the 3,5-dinitrosalicylic acid (DNS) method [13]. The assay was conducted by premixing α-amylase with the extract at varying concentrations (50–200 µg/mL). The reaction was initiated by adding 0.5% starch as a substrate and the mixture was incubated at 37°C for 5 min. After incubation, 2 mL of DNS reagent was added to complete the reaction and the mixture was heated subsequently for 15 min at 100°C. In an ice bath, the reaction mixture was diluted with 10 mL of distilled water. The absorbance was measured at 540 nm to determine α-amylase activity. The

mixture of all chemicals and the enzyme, excluding the test sample, served as a control. The inhibition % was determined using the following formula:

$$\%\text{Inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample}) \times 100}{\text{Absorbance Control}}$$

FTIR Spectroscopic Analysis

The dried leaf powder of the sample was examined for FTIR analysis. The FTIR analysis was conducted with a Perkin Elmer Spectrophotometer, to identify the characteristic peaks and their associated functional groups. The graph obtained was analysed for the presence of functional groups.

Statistical Analysis

All analyses were conducted in triplicate and the data were statistically evaluated and presented as mean (n=3) ± standard deviation (SD).

RESULTS AND DISCUSSION

Percentage Yield of the Extract

C. buxifolius leaves extracted using different types of solvents such as petroleum ether, chloroform, ethyl acetate, ethanol and distilled water, showed that maximum yield was obtained from aqueous extract (8.14%). Followed by aqueous extract ethanol extract (1.69%), chloroform (1.65%), ethyl acetate (1.38%) and Petroleum ether (1.04%). Showed the highest percentage of yield respectively. Consequently, the findings suggest that highly polar solvents are more effective in dissolving the active compounds from leaves of *C. buxifolius.*

Qualitative Phytochemical Screening

Qualitative phytochemical screening serves as an essential preliminary step in identifying bioactive compounds in plants. These tests are simple, inexpensive and rapid, relying mainly on observable color changes that indicate reactions between plant constituents and specific reagents. Such preliminary analyses are valuable for guiding further research, validating traditional medicinal uses and supporting basic quality control in drug development[14].

The preliminary phytochemical screening of *C. buxifolius* leaf powder showed the presence of therapeutic compounds. Based on the color intensity, it was noticed that Carbohydrates, flavonoids, phenolic compounds and glycosides were detected in high intensity (+++) indicating their significant presence. Proteins, alkaloids and amino acids were moderately present (++), while flavanol glycosides, cardiac glycosides, saponins and tannins were found in lower concentrations (+). However, gums and mucilage were absent (-). The varying intensities of these phytochemicals suggest that *C. buxifolius* leaves contain a diverse range of bioactive compounds that may contribute to their potential medicinal properties.

Previous phytochemical investigations of *Cotoneaster* species have demonstrated the occurrence of structurally diverse classes of bioactive compounds, including cyanogenic glycosides, phenol, flavonoids, triterpenoids, phytoalexins and proanthocyanidins, collectively contributing to their distinctive and complex phytochemical profiles[1].

Quantitative Analysis of Primary Metabolites

Determination of Total Carbohydrates, Protein and Amino acid Content in *C. buxifolius* Leaves

The total carbohydrates, protein and amino acid content of *C. buxifolius* leaves are presented in

Table 1. Quantifying carbohydrates is important to understand the nutritional value, energy content and potential therapeutic uses of plant or food sample. The total carbohydrate content of *C. buxifolius* is 15.5 g per 100 g of sample, whereas *Malus domestica* (Rosaceae) contains 12.10 g per 100 g as reported by Arnold and Gramza-Michalowska (2024)[15]. These results showed that *C. buxifolius* possesses a significantly higher carbohydrate content than *M. domestica*, indicating that it has the potential to be a more significant source of energy. This difference underscores potential variations in their nutritional value and possible applications in food and medicinal contexts.

Protein is an essential nutritional component needed throughout life of living things. It contributes and secures growth in infancy, supports muscle development and bone metabolism [16]. The total protein content in *C. buxifolius* is 40.30 g per 100 g, whereas total protein of *C. nummularia* contains 12.4 g per 100 g, as reported by Hafiz Ullah and Lal Badshah (2023)[16]. This indicates that *C. buxifolius* exhibited a higher protein content, approximately 3.25 times more than *C. nummularia*. The high protein content in *C. buxifolius* implies its potential as a valuable dietary protein source, which could be beneficial for food, pharmaceutical and nutraceutical applications.

Amino acids are very much helpful in production of Human Growth Hormone (HGH), strengthening of lean muscles, regulates blood flow, hormonal production, act as vasodilator, maintains chemical balance of various tissues including brain and have been reported to be back bone of healthy life. High cysteine and methionine have been identified to have high antioxidant capacity which helps in reducing the risk of cancer [15]. Total free amino acid content of *C. buxifolius* leaf was 16.24 g Leucine equivalents/ 100 g sample.

Table 1. Quantitative Analysis of Primary Metabolites is *C. buxifolius* Leaves

Primary metabolites	*C. buxifolius* leaf
Total Carbohydrates (g Glucose equivalents/ 100 g sample)	15.55± 3.05
Total Proteins (g BSA equivalents/ 100 g sample)	40.30 ± 0.65
Total free amino acid (g Leucine equivalents/ 100 g sample)	16.24 ± 7.51

Values are mean of triplicate determination (n=3) ± Standard deviation

Quantitative Analysis of Secondary Metabolites:

Determination of Total Phenolics, Tannin and Flavonoid Content of *C. buxifolius* Leaves

Estimation of total phenolic compounds, flavonoid and tannin content of the various extracts of *C. buxifolius* leaves are presented in Table 2. Quantifying phenolic compounds, tannins and flavonoids in plant crude extracts is essential due to their significant bioactive properties (e.g., antioxidant, antimicrobial) and roles in medicinal, nutritional and industrial applications.

The content of total phenolics was determined based on the absorbance reading of different extracts of *C. buxifolius* leaves ranging from 226.5 to 981.99 mg GAE /g. Among all the studied extracts, ethanol extract exhibited the highest value of phenolics (981.99 mg GAE/g extract). Plants with higher phenolic content demonstrate the significant antioxidant activity, showing a direct correlation between phenolic content and antioxidant properties.

The antioxidative properties of polyphenols arise from the capability of the polyphenol-derived

radical to stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions [17]. According to Kicel et al. (2016) [18], the total phenolic content (TPC) for different *Cotoneaster* species showed that TPC in *C. bullatus* (154.3 mg GAE/g extract), *C. zabelii* (129.4 mgGAE/g extract), *C. hjelmqvistii* (124.9 mgGAE/g extract), *C. divaricatus* (119.7 mgGAE/g extract), *C. tomentosus* (51.7 mgGAE/g extract), *C. melanocarpus* (54.8 mgGAE/g extract) and *C. dielsianus* (69.9 mgGAE/g extract). This remarkable difference in phenolic content suggests that *C. buxifolius* may exhibited significantly greater antioxidant potential and bioactive properties than other *Cotoneaster* species. The variations in TPC among *Cotoneaster* species could be attributed to factors such as genetic differences, environmental conditions, extraction methods and geographical variations.

Tannins are polyphenolic compounds with significant biological activities, including antioxidant, antimicrobial and anti-inflammatory effects. Quantification of tannins in plant leaves provides insight into their phytochemical composition, supports correlation with biological activity and aids in standardization and quality control of plant materials[19]. The concentration of the tannins in *C. buxifolius* leaf extracts was measured and the results ranged from 57.4 to 272.8 mg GAE/g. The highest tannin content was observed in the ethanol extract (272.8 mg GAE/g extract).

The total tannin content of the *C. buxifolius* ethanolic extract was (272.8 mgGAE/g), is higher than that reported for other Rosaceae members such as *Potentilla alba* (237.6 mgGAE/g) and *Potentilla fruticosa* (178.7 mgGAE/g) (Augustynowicz, 2023)[20]. This higher concentration may be attributed to species-specific metabolic capacity and environmental factors influencing phenolic biosynthesis. The elevated tannin content highlights the strong antioxidant potential of *C. buxifolius* and supports its pharmacological and traditional relevance as a source of bioactive compounds.

Among the various solvent extracts of *Cotoneaster buxifolius* leaves, the total flavonoid content was found to range between 17.09 and 319.76 mg RE/g extract. This wide variation suggests that the efficiency of flavonoid extraction is strongly dependent on the solvent system employed. The highest level of flavonoid content was observed in the ethanol extracts (319.76mg RE/g extract).

Total flavonoids content (TFC) was significantly higher than those reported for other *Cotoneaster* species by Kicel et al. (2016) [18] *C. integerrimus* contained 13.2 mg/g, *C. nanshan* 9.7 mg/g, *C. splendens* 7.3 mg/g and *C. divaricatus* 7.0 mg/g. These findings demonstrated that considerably greater concentration of flavonoids are present in *C. buxifolius* compared to its congeners. One of the most important classes of secondary metabolites with a wide range of pharmacological and medicinal applications is flavonoids. The anti-oxidative activity of flavonoids is due to various mechanisms such as scavenging of free radicals, chelation of metal ions and copper and inhibition of enzymes responsible for free-radical generation. Depending on their structure, flavonoids are able to scavenge practically all known ROS [21].

Table. 2. Quantification of Total Phenolics, Tannin and Flavonoid Content of *C. buxifolius* leaves

Extracts	Total Phenolics (mg GAE/g extract)	Total Tannins (mg GAE/g extract)	Total Flavonoid (mg RE/g extract)
Petroleum ether	226.5 ± 0.1	57.4 ± 0.78	17.09 ± 0.8
Chloroform	229.32 ± 0.1	61.03 ±0.39	40.82 ± 0.4
Ethyl acetate	777.19 ± 0.4	189.46 ± 0.75	95.22 ± 0.6
Ethanol	**981.99 ± 0.8**	**272.8 ± 0.63**	**319.76 ± 0.8**
Water	440.76 ± 0.5	131.62 ± 0.36	35.89 ± 0.6

GAE- Gallic Acid Equivalents and RE –Rutin Equivalents
*Values are mean of triplicate determination (n=3) ± Standard deviation.

In vitro Antioxidant Assay

DPPH Radical Scavenging Activity

As a persistent free radical, DPPH is frequently used to evaluate an antioxidant compound's potential to neutralize radicals. The lowest IC50 values demonstrated the greatest free radical scavenging activity of the extract. Among the extracts examined, the ethanol extract of leaves (44.35 µg/ mL) exhibited lower IC_{50} values for DPPH radical scavenging activities than the other solvent extracts (Fig 1). The radical scavenging activity of the standard drug rutin was found to be 7.43 µg/ mL.

Compared with *C. melanocarpus* (IC_{50} value 106.41 µg/mL) reported by Veronika and Holzer et al. (2013) [22], *C. buxifolius* showed significant antioxidant properties and radical scavenging activities. This implies that the plant extract includes substances that can donate hydrogen to a free radical in order to eliminate the unpaired electron that causes the radical's reactivity.

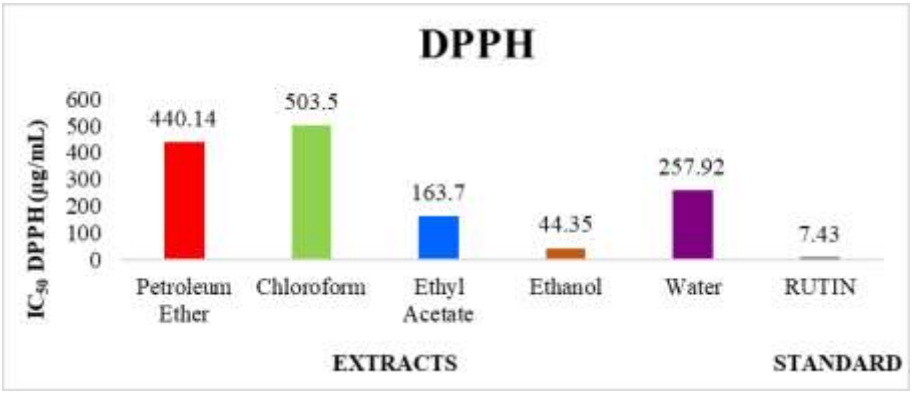


Fig. 1. DPPH Radical Scavenging Activity of *C. buxifolius* leaves extracts.

Values are mean of triplicate determination (n=3)

Phosphomolybdenum Reduction Assay

The phosphomolybdneum assay relies on the antioxidant compounds reducing Mo (VI) to Mo (V). The total antioxidant capacity of different solvent extracts of *C. buxifolius* leaves were recorded and shown in Table 3. Phosphomolybdenum reduction assay is widely used method to determine the total antioxidant capacity. In the current study, the ethanol extract of *C. buxifolius* leaves demonstrated a notably high phosphomolybdenum reducing capacity, recorded at 554.9 mg AAE/g extract. When compared to previously reported result in *Cotoneaster nummularia* it showed values ranging from 56.1 mg AAE/g for the ethyl acetate extract, 177.2 mg AAE/g for the aqueous extract and

161.26 mg AAE/g for the methanolic extract (Kicel et al., 2020)[1]. The comparatively higher activity observed in *C. buxifolius* highlights its potential as a promising natural source of antioxidants.

Ferric Reducing Antioxidant Power (FRAP) Assay

The ethanolic leaf extract of *Cotoneaster buxifolius* exhibited the highest ferric reducing capacity **(**3.13 ± 1.93 mM Fe(II)/g extract**),** which was found to be comparable to that of the standard $FeSO_4 \cdot 7H_2O$ (8.17 mM Fe(II)/g extract) (Table 3).

Kicel et al. (2016) [18] reported the FRAP antioxidants capacities in different *Cotoneaster* sp such as *C. melanocarpus* (0.95 mM Fe (II) /g), *C. integerrimus* (2.04 mM Fe (II) /g) and *C. tomentosus* (0.90 mM Fe (II) /g). According to the results of previous research, study sample *C. buxifolius* has a relatively high ferric reducing power, indicating that they are an electron donor and reduce the amount of oxidized intermediates produced by lipid peroxidation processes.

Table 3: Phosphomolybdenum Reduction and FRAP Assay of *C. buxifolius* Leaves

Extracts	Phosphomolybdenum (mg AAE/g extract)	FRAP mM Fe (II)/g extract)
Petroleum ether	275.6 ±1.48	1.46 ± 1.2
Chloroform	229.21±0.56	1.7 ± 1.45
Ethyl acetate	349.7± 0.65	2.56 ± 0.65
Ethanol	**554.9± 0.65**	**3.13 ± 1.93**
Water	139.1± 0.65	1.41 ± 0.77
AAE	960.38 ± 0.72	-
mM Fe (II)	-	8.17 ± 0.39

AAE - Ascorbic Acid Equivalent and mM Fe (II) - FeSO4·7H2O Equivalent,
*Values are mean of triplicate determination (n=3) ± standard deviation.

Superoxide Radical Scavenging Activity

One of the most harmful radical is superoxide radical species. It damages the cellular components. Moreover, it is a main reason for formation of the ROS [23]. The scavenging capacity of ethanol extract of *C. buxifolius* leaves was found to be 39.8% of inhibition at 100 µg/mL (Figure 2) and followed by ethyl acetate extract (36.52% of inhibition at 100 µg/mL).

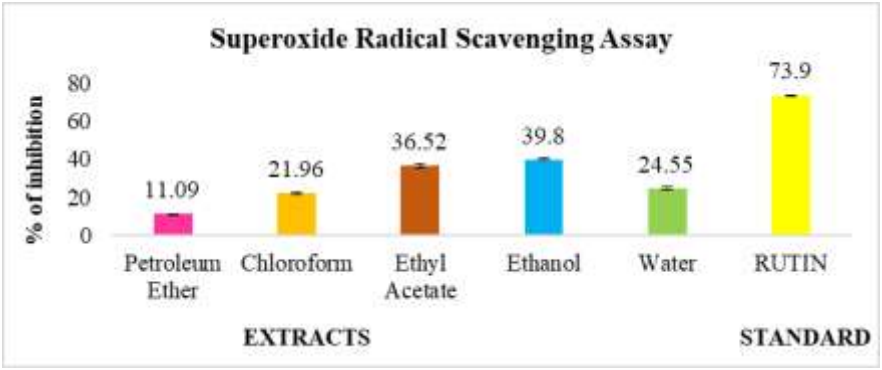


Fig.2. Superoxide Radical Scavenging Activity of *C. buxifolius* Leaves

Values are mean of triplicate determination (n=3).

The superoxide radical scavenging activity of *C. buxifolius* ethanolic leaf extract, evaluated in the present study, yielded an IC_{50} value of 143.39 µg/mL, demonstrating considerable antioxidant capacity. In contrast, Rosaceae family member of *Eriobotrya japonica* leaf extract, as Pawłowska et al. (2023) [24], exhibited a higher IC_{50} value of 244.30 ± 0.38 µg/mL, indicating comparatively

weaker superoxide radical scavenging activity (Pawłowska et al. 2023) [24]. The substantially lower IC_{50} of *C. buxifolius* reflects its greater efficacy in neutralizing superoxide radicals, suggesting its potential as a more potent natural antioxidant agent. Superoxide anion, although a relatively weak oxidant, plays a critical role in the formation of highly reactive and damaging species such as hydroxyl radicals and singlet oxygen, both are significantly contributing to oxidative stress. In the current study, extracts discovered the high superoxide radical scavenging ability. Total flavonoids, which are greater in the ethanolic extracts in our study, are linked to the SO scavenging action.

In- vitro Anti-Diabetic Activity

α- Amylase Inhibition Activity

Pancreatic α-amylase is a crucial digestive enzyme responsible for catalysing the hydrolysis of starch into disaccharides and oligosaccharides, ultimately leading to the release of glucose, which is subsequently absorbed into the bloodstream. Therefore, inhibition of α-amylase activity can effectively reduce the enzymatic degradation of starch within the gastrointestinal tract. As a result, this may lead to decreases in postprandial hyperglycaemia. The inhibition of α-amylase enzyme activity by standard drug Acarbose and *C.buxifolius* leaves are presented in Fig 3. Obtained results revealed that the ethanolic extract (46.76 % at 100 µg/mL) followed by aqueous extract (41.68% at 100 µg/mL) exhibited higher inhibition capacity of α-amylase enzyme in a dose-dependent manner than the other extracts.

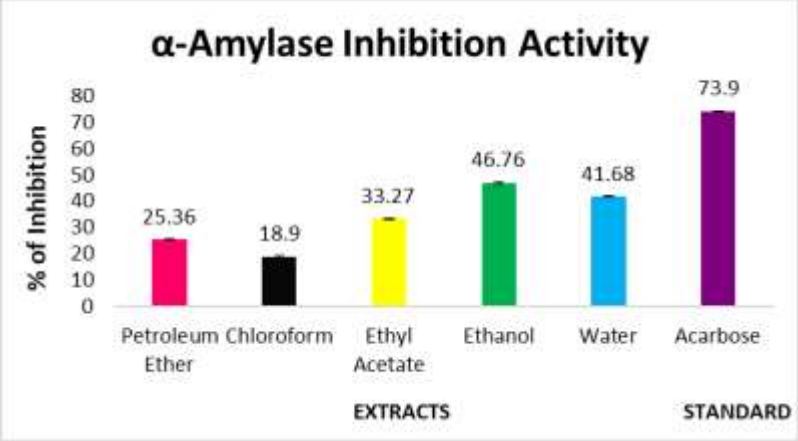


Fig. 3. α-Amylase Inhibition Activity of *C. buxifolius* leaves

*Values are mean of triplicate determination (n=3).

α-amylase inhibition activity is already reported in leaves and fruits of phenolic extracts of *Cotoneaster* species such as *C. bullatus, C. zabelii* and *C. integerrimus.* When compared to a previously reported Rosaceae member, *Pentaphylloides fruticosa*, which demonstrated 31.2% inhibition at a much higher concentration of 0.3 mg/mL (300 µg/mL) (Sales et al. 2012) [25] *C. buxifolius* showed significantly greater inhibitory potency at a lower concentration. This suggests that *C. buxifolius* may possess more effective α-amylase inhibitory constituents, potentially due to a richer or more bioactive profile secondary metabolites. The stronger activity at a lower dose highlights its therapeutic promise as a natural source of α-amylase inhibitors, especially for managing postprandial hyperglycaemia in diabetes.

FTIR Analysis of *C. buxifolius* Leaf

Fourier-transform infrared (FTIR) spectroscopy is employed to identify the functional groups and chemical bonds present in plant extracts. This analysis provides a rapid and non-destructive method to characterize the phytochemical composition, detect bioactive compounds and support the correlation between chemical constituents. According to observed peaks, the functional groups of the components were identified (Fig. 4). The unique absorption band at 3748.94, 3363.25, 2920.66, 2851.24, 1688.37, 1603.52, 1516.74, 1445.39, 1375, 1271.82, 1033.66 and 765.601 cm^{-1} unveiled the presence of the subsequent functional groups, that is Alcohols, Aliphatic primary amines, Alkanes, Aldehyde, Alkene, Nitro compound, Alkanes, Phenol, Aromatic ester, Sulfoxide and Alkyl Halides respectively (Table 4). The respective chemical bonds discovered in the study are O-H stretching, N-H stretching, C-H stretching, C=O stretching, C=C stretching, N-O stretching, O-H bending, C=O stretching, S=O stretching and C-Cl stretching, which may be the functional domains of the bioactives.

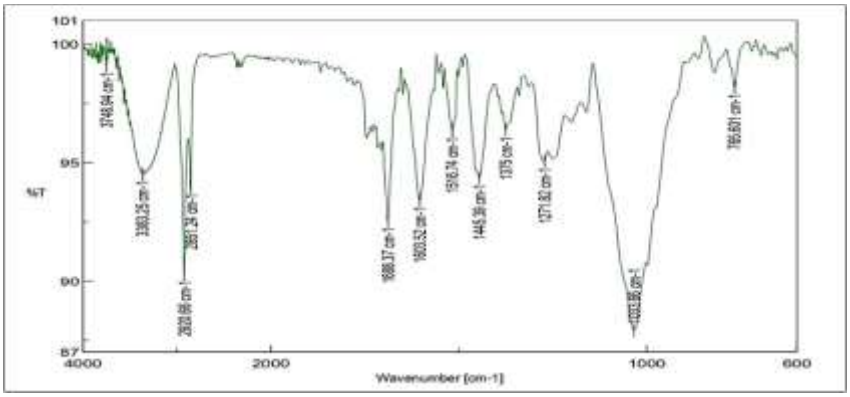


Fig 4: FTIR Spectrum of *C. buxifolius* Ethanolic Extracts of Leaves.

Table 4: FTIR – Functional Groups of *Cotoneaster buxifolius* Ethanolic Extracts of Leaves.

Sr. No.	Peak values (Wave number cm^{-1})	Types of Bond	Functional Groups
1.	3748.94	O-H str.	Alcohols
2.	3363.25	N-H str.	Aliphatic primary amines
3.	2920.66	C-H str.	Alkanes
4.	2851.24		
5.	1688.37	C=O str.	Aldehyde
6.	1603.52	C=C str.	Alkene
7.	1516.74	N-O str.	Nitro compound
8.	1445.39	C-H str.	Alkanes
9.	1375	O-H bending	Phenol
10.	1271.82	C=O str.	Aromatic ester
11.	1033.66	S=O str.	Sulfoxide
12.	765.601	C-Cl str.	Alkyl Halides

CONCLUSION

The present study demonstrated that *Cotoneaster buxifolius* leaves contain a diverse array of bioactive phytochemicals, including phenolics, flavonoids and tannins, which contribute to their notable antioxidant and antidiabetic activities. Among the tested extracts, the ethanolic leaf extract exhibited the highest bioactivity, highlighting ethanol as an effective solvent for extracting active constituents. To the best of our knowledge, this is the first report on the phytochemical composition and bioactivity of this wild and unexplored species. The analytical approaches employed, including Phenolic compound, Tannins and flavonoid quantification and FTIR analysis, providing valuable information for optimizing extraction efficiency and

standardizing herbal preparations. These findings lay a foundation for future research aimed at isolating, identifying and characterizing the active compounds, as well as evaluating their in vivo therapeutic potential. Overall, *C. buxifolius* leaves represent a promising natural source of antioxidants and may serve as a basis for developing plant-based interventions for disease management.

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