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PHYTOCHEMICAL ANALYSIS OF BUXUS SEMPERVIRENS L. LEAF METHANOLIC EXTRACT

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ABSTRACT

The phytochemical investigation of *Buxus sempervirens L.* (Boxwood), commonly known as "Azazer," was done to explore its bioactive constituents and potential pharmacological properties. Soxhlet extraction with methanol was used to isolate a comprehensive range of phytochemicals from the dried leaves of the plant. The methanol extract was investigated for various chemical compounds, such as alkaloids, flavonoids, phenolic compounds, tannins, phytosterols, diterpenes, proteins, amino acids, triterpenoids, fixed oils, and carboxylic acids, through rigorous screening methodologies using standard chemical tests. Results confirmed the existence of several bioactive compounds, with alkaloids, flavonoids, phenolic compounds, tannins, phytosterols, and proteins showing significant presence. Such findings give massive scientific justification of the traditional medicine usage of *Buxus sempervirens L.*, based on the historically established treatment profiles such as curing rheumatism, constipation, malaria, and pneumonia, among others. The diverse phytochemical composition of the methanol extract underscores its promising potential across pharmaceutical, nutraceutical, and therapeutic domains. The comprehensive analysis not only substantiates the plant's traditional medicinal applications but also establishes a molecular foundation for understanding its therapeutic mechanisms. The research ultimately recommends further advanced biochemical and pharmacological investigations to fully explore and leverage the rich molecular complexity of this remarkable plant extract.

KEYWORDS: *Buxus sempervirens L*, Phytochemicals, Methanol, Alkaloids.

INTRODUCTION

Buxus sempervirens L. (Boxwood) known locally as "Azazer" is one of herbs that are used in traditional medicine. This species belonging to the Buxaceae is found mainly in southern and central Europe with a clear division into east and west regions, as well as in North Africa. Traditionally, in folk medicine, preparations of *Buxus sempervirens L*. are used internally for rheumatism and constipation, for malaria and pneumonia, and externally for rashes, hair loss, gout and rheumatic complaints.[1]

The phytochemical screening is considered to be the main process to determine the potential medicinal properties of the plant extract. For the plant species *Buxus sempervirens L*, the use of methanol extraction for extracting bioactive compounds has been studied. This extraction method has proven effective due to its polarity and extraction efficiency for isolating several phytochemical constituents.[2]

The extraction process usually requires selected and prepared plant materials. Fresh leaves of *Buxus sempervirens L*. are collected, usually during optimal seasonal periods like September, then dried and pulverized to maximize extraction efficiency.[3] The standard methodology involves maceration of the dried leaf powder in methanol or hydroalcoholic solutions, followed by filtration and concentration techniques such as rotary evaporation.[4]

The importance of methanol extraction is that it allows the elucidation of the chemical diversity present in the plant, which can be alkaloids, terpenoids, phenolic compounds, and other bioactive molecules. Using systematic extraction protocols, the potential medicinal properties of *Buxus sempervirens L*. can be studied in depth and contribute to the understanding of its pharmacological significance.[5]

MATERIAL AND METHOD

Chemical: Methanol

Equipment: Soxhlet apparatus

Authentication: The *Buxus sempervirens Linn*. Plant were identified and authenticated using herbarium collection at Padmashri vikhe patil college of arts, science and commerce, pravaranagar A/P –Loni kd.Tal- Rahata, Dist-Ahilyanagar.

Extraction: The Soxhlet apparatus is used, placing dried and crushed leaves inside an extraction thimble. Methanol solvent is heated in a round bottom flask and caused to evaporate and rise through the system. The warm solvent vapor reaches a condenser and

cools down, back onto the plant material, where it continuously washes and extracts the bioactive compounds. This cyclic extraction process helps extract all phytochemicals present in the leaves. It generally takes about 16-24 hours at a temperature around 65°C, providing time for the full penetration of solvents and efficient compound recovery. The methanol extract obtained may then be concentrated for further research purposes or even to analyze its phytochemical contents. The method is highly efficient in extracting many metabolites, including alkaloids, flavonoids, and other bioactive compounds from *Buxus sempervirens L*. leaf material.[6,7]

Preliminary phytochemical screening

Detection of alkaloids

1. **Dragendorff's/Kraut's test:** Few mL filtrate + 1-2 mL Dragendorff's reagents, A reddish-brown precipitate was formed indicate the presence of alkaloids.
2. **Hager's test:** Few mL filtrate + 1-2 mL Hager's reagents, A creamy white precipitate was formed indicate the presence of alkaloids.
3. **Mayer's test:** Few mL filtrate + 1-2 drops of Mayer's reagent (Along the sides of test tube),A creamy white/yellow precipitate was formed indicate the presence of alkaloids
4. **Wagner's test:** Few mL filtrate + 1-2 drops of Wagner's reagent (Along the sides of test tube), A brown/reddish precipitate was formed indicate the presence of alkaloids.
5. **Picric acid test:** Few mL filtrate + 3-4 drops of 2% picric acid solution, An orange colour was formed indicate the presence of alkaloids.

Detection of Cardiac Glycosides

1. **Keller-Killani test:** One milliliter of filtrate, 1.5 milliliters of glacial acetic acid, one drop of 5% ferric chloride, and concentrated H2SO4 (along the test tube's side) A, blue coloured solution (in acetic acid layer)was formed shows the presence of glycosides.
2. **Test for Cardenolides:** Extract + pyridine + Sodium nitroprusside + 20% NaOH, A red colour, fades to brownish yellow were formed indicate the presence of glycosides.
3. **Bromine water test:** Plant extract + few mL of bromine water, A yellow precipitate was formed shows the presence of glycosides.
4. **Baljet test:** 2mL extract + a drop of Baljet's reagent, A yellow-orange colour was formed shows the presence of glycosides.

5. **Biuret test:** 2mL filtrate + 1 drop of 2% copper sulphate sol. + 1mL of 95% ethanol + KOH pellets, A pink coloured sol. (in ethanolic layer) was formed shows the presence of glycosides.
6. **Millon's test:** Two milliliters of filtrate + few drops of Millon's reagent, the formation of a white precipitate indicates the presence of glycosides.
7. **Ninhydrin test:** 2mL filtrate + 2 drops of Ninhydrin solution (10mg ninhydrin + 200mL acetone), A purple coloured sol. {Amino acids} was formed shows the presence of glycosides.
8. **Xanthoproteic test:** Plant extract + Few drops of conc. Nitric acid, A yellow coloured sol. was formed shows the presence of glycosides.

Detection of Flavonoids

1. **Alkaline reagent test:** 1mL extract + 2mL of 2% NaOH solution (+ few drops dil. HCl), An intense yellow colour, becomes colourless on addition of diluted acid was formed indicate the presence of flavonoids.
2. **Lead acetate test:** A few drops of a 10% lead acetate solution added to 1 milliliter of plant extract, the presence of flavonoids was indicated by the formation of a yellow precipitate.
3. **Shinoda's test/ Mg-hydrochloride reduction test**: Plant extract, magnesium ribbon fragments, and a few drops of concentrated hydrochloric acid are dissolved in five milliliters of alcohol, A pink to crimson coloured solution {flavonal glycosides} was formed indicate the presence of flavonoids.
4. **Ferric chloride test:** Extract aqueous solution + few drop 10% ferric chloride solution, A green precipitate was formed indicate the presence of flavonoids.

Detection of Phenolic compound

1. **Iodine test:** 1mL extract + few drops of dil. Iodine sol., A transient red colour was formed indicate the presence of Phenolic compound.
2. **Ferric chloride test:** Extract aqueous solution + few drop 5% ferric chloride sol., Dark green/bluish black colour was formed indicate the presence of Phenolic compound.
3. **Gelatin test:** Plant extract is dissolved in 5mL distilled water + 1% gelatin solution +10% NaCl, A white precipitate was formed indicate the presence of Phenolic compound.
4. **Lead acetate test:** Plant extract is dissolved in5mL distilled water + 3mL of10% lead acetate sol., A white precipitate was formed indicate the presence of Phenolic compound.

5. **Ellagic Acid Test:** Plant extract aqueous solution + 5% glacial acetic acid + 5% sodium nitrite solution, Solution turns muddy / Niger brown precipitate was formed indicate the presence of Phenolic compound.

Detection of Tannins

1. **Gelatin test:** Plant extract is dissolved in 5mL distilled water + 1% gelatin solution + 10% NaCl, A white precipitate was formed indicate the presence of Tannins.
2. **Braymer's test:** Three milliliters of distilled water, one milliliter of filtrated water, and three drops of a 10% ferric chloride solution produced a blue-green colour that indicated the presence of tannins.
3. **10% NaOH test:** 0.4mL plant extract + 4mL 10% NaOH + shaken well, Formation of emulsion {Hydrolysable tannins} was formed indicate the presence of Tannins.
4. **Bromine water test:** When 10 ml of bromine water and 0.5 g of plant extract were combined, the bromine became decolored, which is a sign that tannins are present.

Detection of Phytosterols

1. **Salkowski's test:** Filtrate+ few drops of conc. H2SO4 (Shaken well and allowed to stand), Red colour (in lower layer) was formed indicate the presence of Phytosterols.
2. **Libermann-Burchard's test:** 50g of extract is dissolved along the test tube's side in 2mL of acetic anhydride and one or two drops of concentrated H2SO4. The presence of phytosterols was indicated by a variety of color changes.
3. **Acetic anhydride test:** 0.5mL plant extract + 2mL of acetic anhydride + 2mL conc. H2SO4, Change in colour from violet to blue/green was formed indicate the presence of Phytosterols.

Detection of Diterpenes

1. **Copper acetate test:** Plant extract is dissolved in distilled water + 3-4 drops of copper acetate solution, Emerald green colour was formed indicate the presence of Diterpenes.

Detection of Proteins and Amino acids

1. **Biuret test:** 2mL filtrate + 1 drop of 2% copper sulphate sol. + 1mL of 95% ethanol + KOH pellets, A pink coloured sol. (in ethanolic layer) was formed indicate the presence of Proteins and Amino acids.
2. **Millon's test:** 2mL filtrate + few drops of Millon's reagent, A white precipitate was formed indicate the presence of Proteins and Amino acids.

3. **Ninhydrin test:** 2 mL of filtrate plus two drops of a solution of ninhydrin (10 mg ninhydrin plus 200 mL acetone) A sol that is purple in color. The presence of proteins and amino acids is shown by the formation of {Amino acids}.
4. **Xanthoproteic test:** Plant extract + Few drops of conc. Nitric acid A yellow coloured sol. were formed indicate the presence of Proteins and Amino acids.

Detection of Triterpinoides

1. **Salkowski's test:** Filtrate + few drops of conc. H2SO4 (Shaken well and allowed to stand) Golden yellow layer (at the bottom) was formed indicate the presence of Triterpinoides.

Detection of Fixed Oils and Fat

1. **Spot test/ Stain test:** Little quantity of plant extract is pressed in between to filter papers Oil stain on the paper was formed indicate the presence of Fixed Oils and Fat.
2. **Saponification test:** Extract + few drops of 0.5N alcoholic KOH + A drop of phenolphthalein (Heated for 2hr.) Soap formation or partial neutralization of alkali was formed indicate the presence of Fixed Oils and Fat.

Detection of Carboxylic acid

1. **Effervescence test:** 1mL plant extract + 1mL sodium bicarbonate solution, effervescence was formed indicate the presence of carboxylic acid.[8]

RESULTS

Table 1: Preliminary phytoconstituents analysis of methanol extract of Buxus sempervirens L. leaf.

Sr. No.	Phytoconstituents	Test	Methanol extract
1	Alkaloids	Dragendorff's test	+
		Mayer's test	+
		Hager's test	+
2	Flavonoids	Alkaline reagent test	+
		Lead acetate test	+
		Shinoda's test	+
		Ferric chloride test	+
3	Phenolic compounds	Iodine test	+
		Ferric chloride test	+
		Gelatin test	+
		Lead acetate test	+
		Ellagic Acid Test	+
4	Tannins	Gelatin test	+

		Braymer's test	+
		10% NaOH test	+
		Bromine water test	+
5	Phytosterols	Salkowski's test	+
		Libermann- urchard's test	+
		Acetic anhydride test	+
6	Diterpenes	Copper acetate test	+
7	Proteins and Amino acids	Biuret test	+
		Millon's test	+
		Ninhydrin test	+
		Xanthoproteic test	+
8	Triterpinoides	Salkowski's test	+
9	Fixed Oils and Fat	Spot test/ Stain test	+
		Saponification test	+
10	Carboxylic acid	Effervescence test	+

(+) = Presence of phytoconstituents, (-) = Absence of phytoconstituents.

The phytochemical analysis of the methanol extract shows a wide diversity of bioactive compounds, with significant presence of phytoconstituents across multiple chemical classes. Alkaloids were positively identified through Dragendorff's, Mayer's, and Hager's tests, indicating the pharmacological properties of the extract. The flavonoid profile was comprehensively confirmed by alkaline reagent, lead acetate, Shinoda's, and ferric chloride tests, suggesting antioxidant and therapeutic potential. The phenolic compounds and tannins were qualitatively extensively validated using different chemical tests involving iodine, ferric chloride, gelatin, and lead acetate examinations. The extract, therefore, shows the presence of phytosterols by employing Salkowski's and Libermann-Burchard's tests, thus pointing toward its nutritional as well as medicinal value. The presence of proteins and amino acids was confirmed by Biuret, Millon's, Ninhydrin, and Xanthoproteic tests, which showed a complex molecular composition containing nitrogen. Other phytochemicals such as triterpinoids, diterpenes, fixed oils, and fats were systematically detected, which further underlines the biochemical complexity of the extract. The overall positive results from the various chemical tests indicate that this methanol extract contains a rich, multifaceted mixture of bioactive molecules with potential applications in pharmaceutical, nutraceutical, and therapeutic domains. Each positive test furnishes chemical evidence of the molecular diversity of the extract, hence a promising candidate for further advanced biochemical and pharmacological investigations.

DISCUSSION

The phytochemical analysis of the methanol extract of *Buxus sempervirens L*. (Boxwood) revealed a complex and diverse array of bioactive compounds with significant potential for pharmaceutical and therapeutic applications. The comprehensive analysis demonstrated the presence of multiple important phytochemical classes alkaloids, flavonoids, phenolic compound and tannins, phytosterols, protein and amino acids. The study utilized methanol extraction using a Soxhlet apparatus, which is particularly effective for isolating bioactive compounds. Freshly collected leaves were used at appropriate seasonal times of collection, with the plant material being dried, pulverized for extraction using methanol at an approximate temperature of 65°C for 16-24 hours. The plant *Buxus sempervirens L*. presents a richly complex molecular content. Methanol extract suggests promising applications in Pharmaceutical research, Nutraceutical development, Therapeutic interventions *Buxus sempervirens L*, locally known as "Azazer", is traditionally used in medicine for treating conditions like rheumatism, constipation, malaria, and pneumonia. This scientific analysis provides a molecular basis for understanding its traditional medicinal applications. The comprehensive positive results across various chemical tests underscore the extract's potential as a valuable subject for advanced biochemical and pharmacological research.

CONCLUSION

The phytochemical analysis of methanol extract of *Buxus sempervirens L.* (Boxwood) reveals complex and diverse array of bioactive compounds with potential for pharmaceutical and therapeutic applications. The comprehensive analysis demonstrated the presence of multiple important phytochemical classes, such as alkaloids, flavonoids, phenolic compounds, tannins, phytosterols, and proteins and amino acids. The isolation of these bioactive compounds by methanol using Soxhlet apparatus effectively shows that the plant is a potent medicinal agent. Traditionally used for the treatment of rheumatism, constipation, malaria, and pneumonia, this scientific analysis now explains the molecular basis of the medicinal properties of the plant. This rich and diversified molecular composition shows promise in pharmaceutical research, nutraceutical formulation, and therapeutic intervention, hence *Buxus sempervirens L*. an excellent candidate for further advanced biochemical and pharmacological investigations.

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