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Qualitative Phytochemical Screening of *Buddleja crispa* (roots and stem)

Imrad Ali Khan, Sunbal Khan, Hazrat Bilal*

Department of Chemistry, Government Post Graduate Collage Dargai, District Malakand, Khyber Pakhtunkhwa, Pakistan

Correspondence
Hazrat Bilal*: hazratbilal279@gmail.com*

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Abstract

The aim of this study was extraction and Phytochemical Investigation from the Stems and Roots of *Buddleja crispa*. This plant, belongs to the genus *Buddleja* and *Buddleiaceae* family, is a multi-branched shrub or occasionally small tree. This study encounters the phytochemical analysis of *Buddleja crispa* (roots & stems) in ethanol. Primary metabolites like carbohydrate, fixed oils and lipids, reducing sugar, amino acid, hexose sugar and secondary metabolites like as betacyanin, cardic glycoside, flavonides, saponins, tannins,and triterpenoides were found in all Ethanolic extract of *Buddleja crispa*. For phytochemical investigation, the Stems and Roots of the plant were collected from Agra Tehsil Batkhela District Malakand on January 28, 2022. The phytochemical investigation in the EtOH extract of Stems and Roots of *Buddleja crispa* led to the information that most of the biologically active constituents are present except Cyanogenic Glycoside.

KEYWORDS
Phytochemicals, Biologically active, *Buddleiaceae*, *Buddleja*, *Buddleja Crispa.*

1.0 INTRODUCTION

Medicinal plants are increasingly recognized as crucial therapeutic agents for addressing human ailments globally [1]. Traditional medicines derived from natural plants are highly valuable, with "green medicines" being safer and more accessible to the general public compared to expensive synthetic alternatives with more side effects [2]. The successful prevention and treatment of infectious diseases using plant materials have garnered global attention from scientists [3]. Plants harbor medicinally active compounds known as phytochemicals [4]. These compounds, such as flavonoids, lignins, terpenoids, glycosides, and alkaloids, have a significant impact on the human body. Phytochemicals combine with fibers and nutrients in the body, providing protection against diseases and stress-related issues [5]. Bioactive components of medicinal plants act additively, individually, or synergistically to contribute to human health [6]. Phytochemical-rich sources include fruits, vegetables, whole grains, legumes, nuts, seeds, fungi, herbs, and spices [7]. Phytochemicals accumulate in different plant parts, such as roots, leaves, stems, fruits, and flowers [8]. These compounds are classified into primary and secondary metabolites based on their function in plant metabolism. Primary metabolites include carbohydrates, amino acids, proteins, and chlorophyll, while secondary metabolites comprise terpenoids, alkaloids, steroids, and flavonoids [7]. The presence of secondary phytochemicals in the majority of plants signifies various medicinal effects [9].

Traditional medicine, with a history spanning thousands of years, gained recognition in the World Health Organization program about 35 years ago [10].

Pharmaceutical industries and Western researchers have rediscovered the significant contributions of plants to the discovery of new, effective, safe, and profitable therapeutic agents [10]. In-depth studies on the ethnomedical use of plants within specific ethnic groups, conducted through frequent communication in their own language, provide reliable information [11]. It's important to note that extensive knowledge of traditional medicines may be limited to a few community members, and focusing on this group yields greater results [11].

Buddlejaceae, a family of seven genera and nearly 120 species thrives in tropical and warm areas. Commonly known as the Butterfly Bush family. The leaves are opposite or in whorls, sometimes alternate, and simple. Flowers are actinomorphic, bisexual, in terminal or axillary cymes, sometimes paniculate and bracteate, with fruit in it, in the absence of intraxylary phloem and the presence of glandular, stellate, or scaly indumentums [12]. As a cosmopolitan genus of Buddlejaceae, it consists of approximately 100 species in tropical, subtropical, and temperate zones worldwide [13]. The classification of the genus Buddleja has undergone changes by various authors, originally placed in the family Scrophulariaceae [14] and later reclassified in the Loganiaceae [15]. Buddleja species, including Buddleja globosa, Buddleja davidii, Buddleja officinalis, and Buddleia crispa, have been subject to previous studies resulting in the isolation of various compounds, such as glycosides of triterpenes, iridoides, flavonoids, steroids, and aryl esters [11]. Buddleja globosa, known for its wound healing properties, stimulates dermal fibroblast growth and reduces inflammation when applied topically for burns, external and internal ulcers [16]. Buddleia officinalis flowers and flower buds possess antispasmodic, slightly cholagogue, and ophthalmic properties, reducing blood vessel permeability and fragility in the skin and small intestine. They are used in the treatment of various eye problems, gonorrhea, hepatitis, and hernia. A decoction of the leaves is used for collyrium and other conditions [17].

2.0. MATERIALS AND METHODS

2.1. Plant collection

Fresh, Healthy and disease free Stem and Roots of *Buddleja crispa* were collected from the Mountains of Agra Tehsil Batkhela District Malakand on 28, January 2022. The plant were verified and authenticated by Professor Muhammad Ibrahim at the Department of Botany GPGC Dargai Malakand KPK.

2.2 Extraction

The stem and roots were washed with water and dried under shade for 14 days. Washed and air-dried stems and roots were cut into small pieces and powdered in a domestic blender and made ready for the solvent extraction process **(Figure. 1)**.

Figure 1: *Buddleja crispa* Plant in Dried and Powdered form

2.3. Solvent Extraction Process

The extraction process involved the use of EtOH (ethanol) as the solvent. A total of 150 grams of stems and 150 grams of roots were separately soaked in 1500 ml of EtOH and allowed to stand at room temperature for fourteen days (refer to **Figure 2**). Subsequently, the extracts underwent filtration using Whatman filter paper No. 42 (125mm). The resulting extracts were then subjected to evaporation until dryness using a hot water bath maintained at a temperature range of 35°C to 40°C, lasting for duration of 72 hours. Following evaporation, the extracts were stored in a refrigerator at 4°C for future use.

Figure 2: Extraction and filtration process

2.4 Chemicals Used

The following chemicals were used.

H_2SO_4, Chloroform, Ethyl Acetate, Distilled Water, $FeCl_3$, Olive Oil, Conc. & Dil. Hydrochloric acid (HCl), NH_3, $CoCl_2$, (NaOH, Iodine, $CuSO_4$, Ninhydrin, Benzene (C_6H_6), Picric Acid, $NaCO_3$, Concentrated CH_3COOH).

2.5. Reagent Used

Benedict Reagent, Fehling A & B solution, Barfoeds Reagents, Millions Reagents, Wagner Reagent, Selwinofs Reagent, Mayer's Reagent, Hager's Reagents.

2.6. Phytochemical screening

Primary Phytochemicals

2.6.1. CARBOHYDRATE TESTS

2.6.1.1. Molisch's Test

A few drops of Molisch's reagent (naphthol solution in ethanol) were applied to 3 millilitres of the extracted sample. To create a lower layer, concentrated sulfuric acid was then carefully poured down the test tube's side. The presence of carbohydrates was indicated by a purple interfacial ring.

2.6.2. TEST FOR REDUCING SUGAR

2.6.2.1. Benedict's test

Benedict's test solution and an equal amount of the extracted material were placed in a test tube. Warmed in a 5-minute bath of hot water. The colour of the solution might be green, yellow, or red, depending on how much reducing sugar is in the test solution.

2.6.2.2. Fehling's Test

An equal volume of test sample extract was added to 1 mL of Fehling A and Fehling B solution, which was then brought to a boil for one minute. Mixture was cooked for five to ten minutes in a boiling water bath. A brick red and then a yellow PPT were seen at first.

2.6.3. TEST FOR MONOSACCHARIDE

2.6.3.1. Barfoed's Test

The test sample extract and Barfoed's reagent were combined in equal volume. The solution was brought to a boil for one to two minutes and then allowed to cool. The production of a crimson precipitate within a few minutes indicates a positive test.

2.6.4. HEXOSE SUGAR TEST

2.6.4.1. Cobalt Chloride Test

2 mL of cobalt chloride and 3 mL of the test sample extract were combined. It was cooked, and then allowed to cool. Following that, a small amount of NaOH solution was added. The solution appears as either a combination of glucose and fructose (purplish) or greenish blue (glucose) or as an upper layer that is greenish blue and a lower layer that is purplish.

2.6.4.2. Selwinoff's Test

One millilitre of test sample extract and three millilitres of Selwinoff's reagent were cooked in a boiling water bath for one to two minutes, and red coloration was seen.

2.6.5. TEST FOR NON-REDUCING SUGARS

The test solution for Fehling and Benedict does not provide a reaction.

2.6.6. STARCH TEST

2.6.6.1. Iodine Test

Mix 3 millilitres of sample extract with a few drops of diluted iodine solution. The emergence of blue colouring denotes the presence of starch. When boiling, it vanishes and then comes back when cooled.

2.6.7. PROTEIN TEST

2.6.7.1. Biuret Test (General Test)

A 4% NaOH solution was incorporated to a 3 mL test sample extract. Following that, a little amount of 1% $CuSO_4$ solution was added. Colours like violet or pink emerge.

2.6.7.2. Million's Test

5 mL of Million's reagent was combined with 3 mL of sample extract. The white PowerPoint appears. When heated, white PPT becomes brick red or dissolves green solution.

2.6.8. TESTS FOR AMINO ACIDS

2.6.8.1. Ninhydrin Test (General Test)

A boiling water bath was used to cook 3 mL of sample extract and 3 drops of 5% Ninhydrin solution for ten minutes. The colour blue or purplish occurs.

2.6.9. TEST FOR STEROIDS AND PHYTOSTEROLS

2.6.9.1. Sulphuric Acid Test

An equal volume of chloroform and a few drops of strong H_2SO_4 were introduced to one millilitre of plant extract. The occurance of phytosterols is shown by the production of bluish green colour, whereas the existence of steroids is indicated by the formation of a brown ring.

2.6.10. TEST FOR FIXED OIL AND LIPIDS

2.6.10.1. Filter paper Test

A tiny amount of every extract squeezed between two filter sheets and given time to air dry. The existence of fixed oil and fat is indicated by the emergence of an oil stain or grease mark on the filter paper.

2.7. SECONDARY PHYTOCHEMICALS

2.7.1. ANTHRAQUINONE GLYCOSIDES TEST

2.7.1.1 Bontrager's Test

H_2SO_4 was added to 3 mL of the extract dilution of the sample, heated, and filtered. An equal volume of benzene and $CHCl_3$ was introduced to the cooled filtrate. It was thoroughly rattled. Ammonia was injected after the organic layer was separated. Ammoniacal layers become red or pink.

2.7.2.2 Modified Borntrager's Test

5 milliliters of sample extract solution It was added to 5 mL of 5% $FeCl_3$ and 5 mL of Dil. HCl. It was then cooked in a bath of boiling water for an hour. After cooling, benzene was added and well-shaken. After separating the organic layer, an equal amount of diluted ammonia was applied. The ammoniacal layer appears reddish-pink.

2.7.3. CARDIAC GLYCOSIDES TEST

One millilitre of conc. sulfuric acid was placed over five milliliters of each sample extract, which had been treated with two milliliters of glacial acetic acid containing one drop of ferric chloride solution. A dark interference ring signifies a deoxysugar. Caredenolide characteristics. A thin coating may eventually develop a violet ring.

2.7.3. SAPONIN GLYCOSIDES

2.7.3.1. Foam Test

Sample extract and water was shaken gently. Formation of foam was occurred.

2.7.4. CYANOGENETIC GLYCOSIDES

2.7.4.1. Sodium Picrate Test

First, 10% picric acid was absorbed into filter paper. After that, it was dried in a 10% sodium carbonate solution. Subsequently, we take our sample extract and cork it in a conical flask. The filter paper was inserted into the flask through the slit. The filter paper became maroon or brick red.

2.7.5. ALKALOID TEST

2.7.5.1. Hager's test

3 mL of Hager's reagent was added to 1 mL of sample extract. Precipitate with a yellow hue suggests the presence of alkaloids.

2.7.5.2 Wagner's Test

Reddish brown ppt is produced by adding a few drops of Wagner's reagent to 2-3 mL of filtrate.

2.7.5.3. Harborne Method

200 mL of 10% acetic acid was combined with ethanol in a 250 ml conical flask and a small amount of extract was added. The conical flask was kept for a few hours. The reaction mixture as filtered and then concentrated on a water bath to a quarter of its initial volume. Drop by drop, ammonium hydroxide was added until the precipitation stopped.

2.7.6. TANNIN TEST

5 milliliters of the sample extract were extracted, heated in 20 milliliters of chloroform, and then filtered. Following filtering, a small amount of 0.1% ferric chloride was applied, and the brownish color was noted.

2.7.7. SAPONINS TEST

For identification of saponins, a sample extract (5 milliliters) was obtained and combined with 20 milliliters of chloroform in a water bath, and then filtered. Subsequently, 10 milliliters of the filtrates were mixed with 5 milliliters of distilled water and agitated energetically to produce a stable, long-lasting foam. After adding three drops of olive oil and giving it a good shake, it was checked to see whether any emulsion had formed.

2.7.8. PHLOBOTANNINS TEST

For phlobotannins test extract of each sample was boiled with 1% HCl and was then seen for Red precipitate.

2.7.9. FLAVONOIDS TEST

After adding 5 mL of ammonia solution to a part of the chloroform extract filtrate and then conc. H_2SO_4, each

extract was tested for the presence of flavonoids by looking for a yellow tint. Standing causes the golden tint to vanish.

2.7.9. TERPENOIDS TEST

A layer of foam formed when 3 mL of concentrated H_2SO_4 and 2 mL of chloroform were gently added to 5 mL of the sample extract. The development of a rusty-brown color suggested the presence of terpenoids.

3.0 RESULT

The current study conducted on the *Buddleja crispa* shows the occurrence of different therapists. In this study, bioactive compounds of *Buddleja crispa* were qualitatively examined for stem and roots separately and the results are presented in **Tables 1, 2, 3,** and **Table-4** respectively. In this phytochemical analysis, various classes of compounds was identified such as tannins, alkaloids, saponins, flavonoids, terpenoids, etc.

The word positive shows the presence while negative shows the absence of the corresponding phytochemical in the corresponding part extract of the plant.

4.0 DISCUSSION

4.1. *Buddleja crispa* Stem

EtOH extract of *Buddleja crispa* Stem were taken for the comparative study of medicinally active compounds. Carbohydrates, Reducing Sugar, Amino acid, Lipids, Saponins, Tannins, Terpenoids, Anthraquinone Glycosides and Hexose sugar, Cardiac glycoside, Saponins glycosides, Protein, Alkaloids, Flavonoids, Phytosterols and Phlobotannins tests were Positive while tests for Non-reducing sugar, Non-reducing polysaccharides (starch) and Cyanogenetic glycoside were negative.

4.2. *Buddleja crispa* Roots

For the Qualitative Phytochemicals Study EtOH extract of *Buddleja crispa* Roots were taken and different tests were carried out to find out the Primary and Secondary Phytochemicals in the corresponding part. Carbohydrates, Reducing Sugar, Amino acid, Lipids, Saponins, Tannins, Terpenoids, Anthraquinone Glycosides and Hexose sugar, Cardiac glycoside, Saponins glycosides, Protein, Alkaloids, Flavonoids, Phytosterols and Phlobotannins tests were Positive while tests for Non-reducing sugar, Non-reducing polysaccharides (starch) and Cyanogenetic glycoside were negative.

Table 1: Experimental results of Primary Phytochemicals of *Buddleja crispa* stem

S. NO.	Tests		*Buddleja crispa* Stem
Primary phytochemicals			
	Carbohydrates	Molisch's test	Positive
	reducing sugar	Benedict's test	Positive
		Fehling's test	Positive
	Hexose sugar	Cobalt Chloride test	Positive
		Selwinoff's test	Positive
	Non-reducing Sugar	Benedict's test	Negative
		Fehling's test	Negative
	Non-reducing Polysaccharide	Iodine test	Negative
	Protein	Biuret's test	Positive
		Million's test	Positive
	Amino acid	Ninhydrin test	Positive
	Fixed oils and lipids	Filter paper test	Positive

Table-2: Experimental results of Secondary Phytochemicals of *Buddleja crispa* roots.

S. No.	Tests		*Buddleja Crispa* Roots
Primary phytochemicals			
1.	Test for Carbohydrates	Molisch's test	Positive
2.	Test for reducing sugar	Benedict's test	Positive
		Fehling's test	Positive
3.	Test for hexose sugar	Cobalt Chloride test	Positive
		Selwinoff's test	Positive
4.	Test for Non-reducing Sugar	Benedict's test	Negative
		Fehling's test	Negative
5.	Test for Non-reducing Polysaccharide	Iodine test	Negative
6.	Test for Protein	Biuret's test	Positive
		Million's test	Positive
7.	Test for Amino acid	Ninhydrin test	Positive
8.	Test for fixed oils and lipids	Filter paper test	Positive

Table-3: Experimental results of Primary Phytochemicals

of *Buddleja crispa* stem.

S.No.	Tests		*Buddleja Crispa* Stem
Secondary phytochemicals			
1.	Test for Alkaloids	Hager's test	Positive
		Wagner's reagent	Positive
		Mayer's Reagent	Positive
2.	Test for Cardiac Glycoside	Acetic acid test	Positive
3.	Test for Cyanogenic Glycosides	Sodium Picrate test	Negative
4.	Test for Flavonoid	Sulphuric acid test	Positive
5.	Test for Phlobotannins	HCl Test	Positive
6.	Test for Saponins	Foam Test	Positive
7.	Test for Saponins glycoside	Foam Test	Positive
8.	Test for Tannins	Ferric Chloride Test	Positive
9.	Test for phytosterols	Sulphuric acid	Positive
10.	Test for Terpenoids	Chloroform Test	Positive
11.	Test for Anthraquinone Glycoside	Borntrager's Test	Positive
		Modified Borntrager's test	Positive

Table-4: Experimental results of Secondary Phytochemicals of *Buddleja crispa* Roots.

S.No.	Tests		*Buddleja Crispa* Roots
Secondary phytochemicals			
1.	Test for Alkaloids	Hager's test	Positive
		Wagner's reagent	Positive
		Mayer's Reagent	Positive
2.	Test for Cardiac Glycoside	Acetic acid test	Positive
3.	Test for Cyanogenic Glycosides	Sodium Picrate test	Negative
4.	Test for Flavonoid	Sulphuric acid test	Positive
5.	Test for Phlobotannins	HCl Test	Positive
6.	Test for Saponins	Foam Test	Positive
7.	Test for Saponins glycoside	Foam Test	Positive
8.	Test for Tannins	Ferric Chloride Test	Positive
9.	Test for phytosterols	Sulphuric acid	Positive
10.	Test for Terpenoids	Chloroform Test	Positive
11.	Test for Anthraquinone Glycoside	Borntrager's Test	Positive
		Modified Borntrager's test	Positive

5.0. CONCLUSION

The Data collected in this Screening study shows that *Buddleja crispa* is a plant that is well-known for its therapeutic capabilities. These properties have been the subject of several scientific investigations about the plant's makeup (mostly terpenes and phenolic chemicals) and therapeutic characteristics. This makes *Buddleja crispa* a legitimate "medicinal plant" with *the* potential to be used commercially for a range of pharmacologically interesting chemicals. *Buddleja crispa* is still considered a historically used plant with limited production and household use, despite its growing significance in herbal therapy. By comparing the selected parts (Stem and Roots) of *Buddleja crispa* phytochemicals results it was found that both parts (Stem and Roots) of *Buddleja crispa* are rich in most of the phytochemicals while *Buddleja crispa* lacks Cyanogenetic Glycoside.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

[1] M.-C. Castello, A. Phatak, N. Chandra, M. Sharon, Antimicrobial activity of crude extracts from plant parts and corresponding calli of Bixa orellana L, (2002).

[2] R. Nair, S. Chanda, Antibacterial activities of some medicinal plants of the western region of India, Turkish Journal of Biology, 31 (2007) 231-236.

[3] A. Falodun, L. Okunrobo, N.J.A.J.o.B. Uzoamaka, Phytochemical screening and anti-inflammatory evaluation of methanolic and aqueous extracts of Euphorbia heterophylla Linn (Euphorbiaceae), 5 (2006) 529-531.

[4] D. Krishnaiah, T. Devi, A. Bono, R.J.J.o.m.p.r. Sarbatly, Studies on phytochemical constituents of six Malaysian medicinal plants, 3 (2009) 67-72.

[5] F. Afolabi, O.J.I.J.o.A.C. Afolabi, Phytochemical Constituents of Some Medicinal Plants in South West, Nigeria, 4 (2013) 76-78.

[6] K. Schütz, R. Carle, A.J.J.o.e. Schieber, Taraxacum—a review on its phytochemical and pharmacological profile, 107 (2006) 313-323.

[7] P.J.T.I.J.o.E. Dhawale, Science, Phytochemical Analysis of Some Medicinal Plants from Yavatmal District (Ms) India, 2 (2013) 65-66.

[8] E.L. Gibson, J. Wardle, C.J.J.A. Watts, Fruit and vegetable consumption, nutritional knowledge and beliefs

in mothers and children, 31 (1998) 205-228.
[9] K.J.K.s.f. Mathai, nutrition,, t.e. diet therapy, ed. LK Mahan, S. Escott-Stump, Nutrition in the adult years, 271 (2000) 274-275.
[10] R. Pistorius, J.J.B. van Wijk, D. Monitor., Biodiversity prospecting: Commercializing genetic resources for export. Prospeccionando la biodiversidad: comercializando los recursos genéticos para la exportación, (1993) 12-15.
[11] B. Atsbeha, Phytochemical Investigation On The Leaves Of Buddleja Polystachya (Anfar), In, 2011.
[12] N. Sultana, M. Akhter, R.A. Khan, N. Afza, R.B. Tareen, A.J.N.P.R. Malik, Nematicidal natural products from the aerial parts of Buddleja crispa, 24 (2010) 783-788.
[13] G. Chen, W.-B. Sun, H.J.B.J.o.t.L.S. Sun, Ploidy variation in Buddleja L.(Buddlejaceae) in the Sino-Himalayan region and its biogeographical implications, 154 (2007) 305-312.
[14] S.T.J.J.o.t.H.o.B. Scharf, Identification keys, the "Natural Method," and the development of plant identification manuals, 42 (2009) 73-117.
[15] P.J.F.M.-S. Leenhouts, Spermatophyta, Loganiaceae, 6 (1960) 293-387.
[16] Y.-H. Liao, P.J. Houghton, J.J.J.o.N.P. Hoult, Novel and known constituents from Buddleja species and their activity against leukocyte eicosanoid generation, 62 (1999) 1241-1245.
[17] P.J. Houghton, A.Y. Mensah, N. Iessa, L.Y.J.P. Hong, Terpenoids in Buddleja: relevance to chemosystematics, chemical ecology and biological activity, 64 (2003) 385-393.