A comprehensive phytochemical, biological, and toxicological studies of roots and aerial parts of *Crotalaria burhia* Buch.-Ham: An important medicinal plant

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This study was designed to seek the phytochemical analysis, antioxidant, enzyme inhibition, and toxicity potentials of methanol and dichloromethane (DCM) extracts of aerial and root parts of *Crotalaria burhia*. Total bioactive content, high-performance liquid chromatography-photodiode array detector (HPLC-PDA) polyphenolic quantification, and ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) analysis were utilized to evaluate the phytochemical composition. Antioxidant [including 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), 2,2′-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ferric reducing antioxidant power assay (FRAP), cupric reducing antioxidant capacity CUPRAC, phosphomolybdenum, and metal chelation assays] and enzyme inhibition [against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), α-glucosidase, α-amyrase, and tyrosinase] assays were carried out for biological evaluation. The cytotoxicity was tested against MCF-7 and MDA-MB-231 breast cell lines. The root-methanol extract contained the highest levels of phenolics (37.69 mg gallic acid equivalent/g extract) and flavonoids (83.0 mg quercetin equivalent/g extract) contents, and was also the most active for DPPH (50.04 mg Trolox equivalent/g extract) and CUPRAC (139.96 mg Trolox equivalent/g extract) contents.
equivalent /g extract) antioxidant assays. Likewise, the aerial-methanol extract exhibited maximum activity for ABTS (94.05 mg Trolox equivalent/g extract) and FRAP (64.23 mg Trolox equivalent/g extract) assays. The aerial-DCM extract was noted to be a convincing cholinesterase (AChE: 4.01 and BChE; 4.28 mg galantamine equivalent/g extract), and α-glucosidase inhibitor (1.92 mmol acarbose equivalent/g extract). All of the extracts exhibited weak to modest toxicity against the tested cell lines. A considerable quantities of gallic acid, catechin, 4-OH benzoic acid, syringic acid, vanillic acid, 3-OH-4-MeO benzaldehyde, epicatechin, p-coumaric acid, rutin, naringenin, and carvacrol were quantified via HPLC-PDA analysis. UHPLC-MS analysis of methanolic extracts from roots and aerial parts revealed the tentative identification of important phytoconstituents such as polyphenols, saponins, flavonoids, and glycoside derivatives. To conclude, this plant could be considered a promising source of origin for bioactive compounds with several therapeutic uses.

KEYWORDS
Crotalaria burhia, secondary metabolites, antioxidant, enzyme inhibition, toxicity

Introduction

Plants are genetically very diverse and vital to human existence, shelter, food, and medicine. Among plants, the study of medicinal plants has gained worldwide attention in recent years. A substantial amount of research demonstrates the intriguing potential of medicinal plants employed in traditional, complementary, and alternative methods of treating human ailments (Fitzgerald et al., 2020; Erdinc et al., 2021; Tamer et al., 2021). The investigation of medicinal plants as a unique source of enzyme inhibitors, natural antioxidant components, and treatments for a variety of common illnesses has attracted considerable interest (Phumthum et al., 2018). Phytochemicals, also known as secondary metabolites, are bioactive plant molecules and the source of the majority of currently accessible pharmaceuticals. 77% of antibiotics and 547 medicines approved by the FDA by the end of 2013 were derived from natural products, according to a survey (Patridge et al., 2016). Natural products play a major role in medication development; therefore, screening plants for substantial active ingredients can be viewed as a first step toward producing more effective treatments against a broader range of ailments (Bibi Sadeer et al., 2022). Herbal applications are now a rapidly expanding market, with the goal of creating new pharmaceutical and nutraceutical materials with herbal ingredients. Lifestyle diseases such as obesity, cancer, and diabetes mellitus are to blame for the current state of affairs (Ceylan et al., 2016; Yener et al., 2018).

Crotalaria belongs to the family Fabaceae. Approximately 700 species are make up this family disseminated throughout the world's tropical and subtropical regions (Lewis, 2005). In the desert regions of West Pakistan, India, and Afghanistan, C. burhia, or Khip, is found as a shrub and fibrous plant. The ancient Indian Ayurvedic system, identified this plant as having great medicinal potential. Anticancer and soothing properties are found in the leaves, roots, and branches of C. burhia, while fresh plant juice can be used to treat eczema, gout, hydrophobia, pain, and edema. Roots extract with sugar is used to alleviate chronic kidney pain and to treat typhoid fever. It has a wide range of medical properties (Talaviya et al., 2018), Cooling medication can be made from the plant’s leaves, branches, and roots. Gout, eczema, hydrophobia, pain and swelling, wounds and cuts, infection, renal pain, stomach disorders, rheumatism, and joint pain can all be treated using plant juice in traditional medicine (Katewa and Galav, 2006; Sandeep et al., 2010; Bibi et al., 2015). There are several active compounds in this plant, including triterpenoids, flavonoids, anthraquinones, phenols, polyphenols, steroids, alkaloids, and tannins (Kataria et al., 2011; Kumar et al., 2011; Bibi et al., 2015). Additionally, C. burhia’s antibacterial, anti-inflammatory, and antinociceptive properties are supported by its traditional applications (Kataria et al., 2010; Kataria et al., 2012; Soni, 2014; Talaviya et al., 2014; Bibi et al., 2015). Crotalaria burhia is a highly important medicinal plant used to treat different ailments. Some researchers also mentioned that the whole plant, as well as its different parts like its branches, roots, leaves, and stem applied for the cure of diseases (Talaviya et al., 2018). Fresh plant juices have magical ethnobotanical values and are reported to treat different disorders. Crotalaria burhia is a valuable plant used to treat cancer, infections, pain, swelling, inflammation, hydrophobia, and skin diseases (Kataria et al., 2010). This plant is well known for the useful...
cure of general contaminations in the Thal Desert of Punjab (Niaz et al., 2013). Previous literature exposed that it is also utilized as a good soil binder, as food for goats, and in the desert to make sheds for animals and ropes (Sonì, 2014). Some phytochemical studies reported the isolation of secondary metabolites from *Crotalaria burhia* are identified as toxicarol, elliptone, rotenone, sumatrol, deguelin, and tephrosin (Uddin and Khanna, 1979), crotalarine (Ali and Adil, 1973), crospermepine (Ahmad and Fatima, 1986), quercetin, β-sitosterol (Sonì, 2014). However, many species of the *Crotalaria* genus are yet to be explored scientifically.

Polyphenol compounds, which include flavonoids and phenolic acids, are widely distributed throughout the plant kingdom. Over 6,000 different flavonoid species have been discovered so far. In the fight against microbial and insect attacks, they play an important role (Boğa et al., 2016; Bouhafsoun et al., 2018; Bakir et al., 2020). The biological activities of *C. burhia*, a species of the *Crotalaria* genus, was examined in this study with regard to enzymes targeted for the treatment of diabetes type II, Alzheimer’s disease, and skin hyperpigmentation problems. Methanol and DCM were assessed in terms of mg EDTAE/g extract. The details of all assays was measured in terms of Trolox equivalents (mg TE/g extract) while the metal chelating activity was quantified phenolic standards are provided in “Supplementary Material” section. The gradient profiles and calibration parameters of the quantified phenolic standards are provided in Supplementary Tables 1, 2, respectively.

### Materials and methods

#### Plant material and extraction

Dr. H. Waris, Taxonomist of the Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur, recognized *C. burhia* aerial and root parts obtained from Bahawalpur, Pakistan. For future reference, the herbarium of the Department of Pharmacy and Alternative Medicine, also deposited a voucher specimen number. For 15 days, the plant material was kept in the shade to dry. Using a combination of DCM and methanol, the powdered dried plant was extracted over the course of 72 h and further concentrated using rotary evaporator.

#### Phytochemical composition

##### Total bioactive contents

Standard Folin-Ciocalteu and aluminum chloride techniques (Slinkard and Singleton, 1977; Zengin et al., 2016) with minor modifications were used to assess the total phenolic (TPC) and flavonoid (TFC) concentrations. Gallic acid equivalents (mg GAE/g extract) and quercetin equivalents (mg QE/g extract) were used to measure phenolic and flavonoid content, respectively.

##### High-performance liquid chromatography-photodiode array detector polyphenolic quantification

High-performance liquid chromatography-photodiode array detector (HPLC-PDA) analysis was used to determine the presence of 22 distinct polyphenolic standards in each sample. Waters liquid chromatograph with a model 600 solvent pump and a 2996 PDA detector was used for the analysis. The data was collected using Empower v.2 Software (Waters Spa, Milford, MA, United States) (Locatelli et al., 2017). The details of HPLC instrumentation are provided in “Supplementary Material” section. The gradient profiles and calibration parameters of the quantified phenolic standards are provided in Supplementary Tables 1, 2, respectively.

##### Ultra-high performance liquid chromatography-mass spectrometry analysis

RP-UHPLC-MS was used to profile secondary metabolites. An Agilent 6,520 was used to perform UHPLC-MS analysis of methanolic extracts of aerial and root portions (negative ionization mode) on the Agilent 1,290 Infinity LC system (Khurshid et al., 2019). In order to make some tentative predictions about the presence of various secondary metabolites in the samples, we turned to the METLIN database. The details of UHPLC-MS instrumentation are provided in “Supplementary Material” section.

#### Biological activities

##### Antioxidant assays

According to already adopted methods by Grochowski et al. (2017), DPPH and ABTS radical scavenging, reducing power (FRAP, CUPRAC), total antioxidant capacity (phosphomolybdenum), and metal chelating power of the investigated extracts were evaluated. The antioxidant activity of all assays was measured in terms of Trolox equivalents (mg TE/g extract) while the metal chelating activity was assessed in terms of mg EDTAE/g extract. The details of antioxidant assays are provided in “Supplementary Material” section.
Enzyme inhibition assays

The enzyme inhibition potential of plant extracts against cholinesterases (AChE and BChE), tyrosinase, α-amylase, and α-glucosidase was evaluated using previously established in vitro standard methods (Grochowski et al., 2017; Mollica et al., 2017). Galantamine equivalents per gram of extract (GALAE/g) were used to measure AChE and BChE inhibitory activities. On the other hand, millimoles of acarbose equivalents (ACAE/g) and milligram of kojic acid equivalents (KAE/g) were used to measure inhibition of α-amylase, α-glucosidase, and tyrosinase, respectively. The details of enzyme inhibition assays are provided in "Supplementary Material" section.

Cytotoxicity assay

Using the previously published approach, the cytotoxicity of the tested products was assessed against two breast cancer cell lines, MDA-MB 231 and MCF-7 cells, using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Nemudzivhadi and Masoko, 2014). The cell viability percentage (%) was calculated.

Statistical analysis

Three separate experiments were conducted for each of the assays. Mean standard deviation was used to express results (SD). SPSS v.17.0 was employed for data analysis. ANOVA and Tukey’s test were used to examine the differences between the means. Statistical significance was defined as a p-value of 0.05 or less. A link between bioactive content and evaluated biological assays was obtained using PCA and Pearson linear correlation.

Results and discussion

Phytochemical profiling

When it comes to plant secondary metabolites, phytochemicals, such as phenols and flavonoids, are regarded to be the most bioactive secondary metabolites (Rahman et al., 2018). Table 1 lists the TPC and TFC values of methanol and DCM extracts of C. burhia's aerial and root portions, respectively. The methanolic root extract had the highest TPC concentration (37.69 mg GAE/g), whilst the DCM aerial extract had the lowest (27.62 mg GAE/g). The flavonoid content determination followed a similar trend to that of the TPC, with TFC values of 83.11 and 12.64 mg QE/g extract for both methanol root and DCM aerial extracts, respectively.

Similarly, HPLC-PDA polyphenolic quantification was performed in order to quantify the phenolic standards in the studied extracts and the results are presented in Table 2, while, the HPLC-PDA chromatograms of the quantified phenolics in the tested extracts are given in Supplementary Figures 1, 2.
In comparison to the other extracts, *C. burhia* methanol root extract comprised a significant quantity of phenolics (4.28 µg/mg), with the highest amounts of epicatechin (0.71 µg/mg extract) and *p*-coumaric acid (0.68 µg/mg extract), while rutin (0.33 µg/mg extract) was quantified in lesser amount. Likewise, aerial methanol extract presented the highest quantities of epicatechin (1.89 µg/mg extract), while DCM root extract displayed the lowest amounts of carvacrol (0.65 µg/g extract).

Both roots and aerial DCM extracts accounted for the least amounts of phenolic standards (0.65 and 0.36 µg/g extract, respectively), which could be due to the extracts being nonpolar. Further investigations of plant extracts/fractions can be done to separate bioactive compounds with potentially important functions as a result of this phenolic profiling.

Additionally, methanolic extracts of *C. burhia* roots and aerial parts were subjected to UHPLC-MS analysis in order to get thorough profiles of individual secondary metabolites. Figures 1A,B depict standard total ion chromatograms with mass spectrometric peaks for both extracts. Tables 3, 4 give a preliminary list of secondary metabolites found in aerial and root extracts, respectively. A total of 36 distinct secondary metabolites were detected in the methanolic aerial extract. A preliminary analysis of the root extract identified 53 distinct chemicals. Majority of the compounds belonged to phytoconstituents’ phenols, flavonoid, saponin, coumarin, and glycoside classes. Polyphenols, notably flavonoids and coumarins, have been discovered to possess a wide range of health benefits, including antibacterial, enzyme inhibitory and antioxidant capabilities (Dilworth et al., 2017), whereas glycosides, tannins, alkaloids, and resins have been shown to have antibacterial activities (Rascon-Valenzuela et al., 2017). According to our research, this is the first time this plant has been profiled in such detail.

**Antioxidant potential**

Metabolic processes typically produce reactive oxygen species (ROS). Excessive accumulation of ROS causes tissue injury and inflammation by damaging fatty acids, DNA, and proteins. As a result of these illnesses, plant extracts have been examined for their possible function in reducing the oxidative stress burden (Zengin et al., 2022).

Antioxidant activity of *C. burhia* extracts was tested using six different assays, the findings of which may be found in Table 1. To sum up, it was shown that the roots and aerial methanolic extracts had the highest radical scavenging and reducing power assays’ maximum values. Bioactive components with reducing power and anti-oxidant activity have been shown to have a favorable correlation with the amount of phenols and flavonoids found in this extract (Khan et al., 2019). Antioxidant activity was found in phenolic...
compounds quantified through HPLC-PDA, including 4-OH benzoic acid, vanillic acid, syringaldehyde, p-coumaric acid, and carvacrol (Verma et al., 2008). As mentioned in Table 1, the root-methanol extract was the most active for DPPH radical scavenging (50.04 mg TE/g extract) and CUPRAC reducing power potential (139.96 mg TE/g extract). Likewise, the aerial-methanol extract exhibited maximum ABTS radical scavenging (94.05 mg TE/g extract) and FRAP reducing power potential (64.23 mg TE/g extract). The DCM aerial extract exhibited the highest potential for phosphomolybdenum assay at 60.46 mg TE/g and metal chelation activity at 2.24 mg EDTAE/g. Previous studies have shown that this plant has significant antioxidant activity which validates our current findings (Talaviya et al., 2014; Ahmed, 2018). Rutin and naringenin, two important flavonoids with antioxidant potential, were also found in the current study’s HPLC polyphenol quantification and UHPLC-MS analysis (Yang et al., 2008; Cavia-Saiz et al., 2010).

**Enzyme inhibition activities**

Enzyme inhibition is gaining popularity as a therapeutic technique for various global health challenges, including type 2 diabetes, neurodegenerative diseases, and dermatological disorders. This phenomenon illustrates the strategy of inhibiting certain enzymes from treating specific diseases. Neurodegenerative diseases like Alzheimer’s and Parkinson’s have been linked to butyrylcholinesterase (BChE) and Acetylcholinesterase (AChE) (Zengin et al., 2018). Some research has shown that isolated compounds and plant extracts can both inhibit cholinesterase activity (Ballard et al., 2005). Galantamine, an alkaloid extracted from the Galanthus woronowii plant, is one example. Treatment for mild to moderate Alzheimer’s disease with the AChE inhibitor galantamine (Colovic et al., 2013). Previously, significant AChE inhibition potential has been reported in ethanolic extract of C. hebecarpa leaves (IC$_{50}$: 208.6 µg/mL) (Rao et al., 2017). As presented in Table 5, the aerial DCM aerial showed maximum inhibition for AChE (4.01 mg GALAE/g extract) and BChE (4.28 mg GALAE/g extract). While, DCM root extract and methanolic aerial extract displayed the lowest inhibition potential against AChE and BChE (2.07 and 2.93 mg GALAE/g extract), respectively.

The enzyme tyrosinase catalyzes human melanin biosynthesis, also known as melanogenesis, a physiological process that results in the production of melanin (Muddathir et al., 2017). Considering that the inhibition of tyrosinase activity can control melanin formation, dermatological
Hyperglycemia occurs when the pancreas produces less insulin or the cells’ insulin sensitivity decreases. According to previous studies, different phenolics and flavonoids have been shown to have anti-tyrosinase properties, which may explain why the methanolic extract rich in phenolic and flavonoid compounds was found active against mushroom tyrosinase (Zielinska et al., 2017; Choi et al., 2021). Significant tyrosinase inhibition potential of ethanolic extracts of C. hebecarpa shoots to show moderated tyrosinase inhibition (16.12 and 22.45%) at 1 mg/mL (Ketprayoon and Chaicharoenpong).

Similarly, another study reported the methanol and aqueous extracts of C. burhia shoots to show moderated tyrosinase inhibition (16.12 and 22.45%) at 1 mg/mL (Ketprayoon and Chaicharoenpong).

Tyrosinase catalyzes the decomposition of phenolic compounds, which results in undesirable color and taste (Zaidi et al., 2014). C. burhia methanol aerial extract showed maximum tyrosinase inhibition, i.e., 131.72 mg KAE/g extract. In comparison, the methanolic root extract showed inhibition of 128.51 mg KAE/g extract, followed by DCM aerial and DCM root extracts124.95 and 120.76 mg KAE/g extract, respectively (Table 5). According to previous studies, different phenolics and flavonoids have been shown to have anti-tyrosinase properties, which may explain why the methanolic extract rich in phenolic and flavonoid compounds was found active against mushroom tyrosinase (Zielinska et al., 2017; Choi et al., 2021).

Significant tyrosinase inhibition potential of ethanolic extract of another Crotalearia species C. kebecarpa (IC50: 40.15 μg/mL), has been reported previously (Rao et al., 2017). Similarly, another study reported the methanol and aqueous extracts of C. juncea showed to show moderated tyrosinase inhibition (16.12 and 22.45%) at 1 mg/mL (Ketprayoon and Chaicharoenpong).

**TABLE 3** UPHLC-MS analysis tentative identification of the secondary metabolites from C. burhia methanol extract (negative ionization mode).

| No. | RT (min) | Mol. mass | Tentative identification | Chemical formula | Compound class | B. peak (m/z)  |
|-----|----------|-----------|-------------------------|------------------|----------------|---------------|
| 1   | 0.643    | 216.0412  | Isobergaptene           | C12 H4 O4        | Coumarin       | 215.0412      |
| 2   | 7.182    | 294.1315  | Ethyl (S)-3-hydroxybutyrate glucose | C12 H2 O6 | Glycosides | 293.1315      |
| 4   | 7.635    | 640.1647  | Isorhamnetin 3-glucosyl-(1-6)-galactoside | C28 H3 O15 | Flavonoid | 639.1647      |
| 5   | 7.747    | 154.0265  | 3,4-Dihydroxybenzoic acid | C7 H4 O4 | Antioxidant | 153.0265      |
| 6   | 7.759    | 328.0796  | bergenin                | C14 H16 O6 | Phyto | 327.0796      |
| 7   | 7.792    | 432.1279  | Apioxyglucosyl 4-hydroxybenzoate | C18 H14 O12 | Glycoside | 431.1279      |
| 8   | 8.027    | 682.1747  | Isorhamnetin 3-(6′-acetylglucosyl)(1-3)-galactoside | C26 H34 O18 | Flavonoid | 681.1747      |
| 9   | 8.482    | 226.1206  | 12-hydroxyjasmonic acid | C12 H12 O4 | Carboxylic acid | 225.1206 |
| 10  | 8.509    | 330.1307  | (±)-3-(4-Hydroxyphenyl)-1,2-propanediol 4'-O-glucoside | C15 H22 O8 | Phenolic glycosides | 329.1307 |
| 11  | 8.642    | 218.1154  | 3-hydroxy-sebacic acid | C10 H8 O5 | Fatty acids | 217.1154      |
| 13  | 9.35     | 286.0482  | 5,7,2',3' Tetrahydroxyflavone | C15 H10 O6 | Flavone | 285.0482      |
| 15  | 9.864    | 270.0534  | Demethylhexasin         | C15 H10 O5 | Flavonoid | 269.0534      |
| 17  | 10.039   | 300.064   | Kaempferide             | C16 H12 O6 | Flavone | 299.064       |
| 19  | 10.249   | 200.1047  | Decenedioic acid        | C10 H16 O4 | Fatty acids | 199.1047      |
| 20  | 10.42    | 254.0581  | 7,4'-Dihydroxyflavone   | C15 H10 O5 | Flavone | 253.0581      |
| 21  | 10.509   | 286.0479  | 5,7,2',3' Tetrahydroxyflavone | C15 H10 O5 | Flavone | 285.0479      |
| 22  | 10.917   | 268.0373  | Coumestrol              | C12 H7 O4 | Phytoestrogen | 267.0373 |
| 23  | 11.211   | 298.0478  | 8-Methoxycoumestrol     | C16 H10 O5 | Coumestans | 297.0478      |
| 24  | 11.45    | 624.2635  | Kanoxosides D           | C27 H24 O16 | Glycoside | 623.2635      |
| 26  | 11.574   | 314.079   | Luteolin 5,3'-dimethyl ether | C17 H14 O5 | Flavonoid | 313.079       |
| 27  | 11.815   | 370.1053  | Neouralanol             | C20 H12 O2 | Flavonoid | 369.1053      |
| 28  | 11.877   | 354.1105  | 2,3-Dehydrokivitone     | C20 H18 O6 | Iso flavone | 353.1105      |
| 29  | 11.883   | 288.2301  | 9,16-dihydroxy-palmitic acid | C16 H12 O4 | Hydroxy fatty acid | 287.2301 |
| 30  | 12.137   | 562.2627  | 19-Hydroxyvinzeylanol 19-glicoside | C26 H14 O13 | Glycoside | 561.2627 |
### TABLE 4 UPHLC-M5 analysis tentative identification of the secondary metabolites from C. burhia root methanol extract (negative ionization mode).

| No. | RT (min) | Mass       | Tentative identification                                                                 | Chemical formula | Compound class | B. peak (m/z) |
|-----|----------|------------|------------------------------------------------------------------------------------------|------------------|----------------|---------------|
| 1   | 7.794    | 432.1273   | Apioxyglucosyl 4-hydroxybenzoate                                                         | C_{16}H_{24}O_{12} | Glycoside       | 431.1273      |
| 2   | 8.287    | 207.0894   | Phenylpropionylglycine                                                                    | C_{15}H_{15}NO_{3} | Acyl glycine    | 208.0894      |
| 3   | 8.49     | 462.1168   | Tricin 4’-apioside                                                                        | C_{22}H_{22}O_{11} | Flavone         | 461.1168      |
| 4   | 8.871    | 416.1103   | 3’,4’-Dihydroxyflavone 4’-glucoside                                                       | C_{21}H_{20}O_{9} | Flavone         | 415.1103      |
| 5   | 9.213    | 372.1214   | 7,8,3’,4’,5’-Pentamethoxyflavone                                                          | C_{20}H_{20}O_{7} | Flavone         | 371.1214      |
| 6   | 9.351    | 286.0481   | 5,7,2’,3’-Tetrahydroxyflavone                                                             | C_{15}H_{10}O_{6} | Flavone         | 285.0481      |
| 7   | 9.507    | 370.1056   | Neouralenol                                                                               | C_{20}H_{10}O_{7} | Flavone         | 369.1056      |
| 8   | 9.614    | 406.0905   | 5,6,3’,5’-Tetrahydroxy-3,7,8,4’-tetramethoxyflavone                                        | C_{19}H_{14}O_{10} | Flavonoids      | 405.0905      |
| 9   | 9.856    | 270.0536   | Demethylflaxinin                                                                         | C_{15}H_{10}O_{5} | Isolavone       | 269.0536      |
| 10  | 9.942    | 138.0316   | p-Salicylic acid                                                                          | C_{7}H_{4}O_{3}   | Phenol          | 137.0316      |
| 11  | 10.034   | 300.0636   | Kaempferide                                                                               | C_{20}H_{12}O_{6} | Flavone         | 299.0636      |
| 12  | 10.25    | 200.1051   | Decenedioic acid                                                                          | C_{19}H_{10}O_{4} | Phytol          | 199.1051      |
| 13  | 10.358   | 584.2616   | Pubescenol                                                                                | C_{22}H_{26}O_{10} | Withanolide     | 583.2616      |
| 14  | 10.424   | 254.0584   | 7’,4’-Dihydroxyflavone                                                                     | C_{15}H_{10}O_{4} | Flavone         | 253.0584      |
| 15  | 10.533   | 284.0683   | Texasin                                                                                   | C_{15}H_{12}O_{3} | Phytol          | 283.0683      |
| 16  | 10.756   | 390.0955   | 5,7,2’-Trihydroxy-3,6,4’,5’-tetramethoxyflavone                                            | C_{16}H_{16}O_{3} | Flavone         | 389.0955      |
| 17  | 10.822   | 354.1103   | 2,3-Dehydrokrivotone                                                                       | C_{20}H_{16}O_{6} | Phytol          | 353.1103      |
| 18  | 10.921   | 268.0373   | Coumestrol                                                                                | C_{15}H_{14}O_{3} | Coumestans      | 267.0373      |
| 19  | 11.214   | 454.1632   | 5,2’,4’,5’-Tetrahydroxy-3-(3-hydroxy-3-methylbutyl)-6”-dimethylpyranol[2”’,3’’,7’’,8’’]flavone | C_{25}H_{20}O_{6} | Flavone         | 453.1632      |
| 20  | 11.217   | 298.048    | 8-Methoxycoumestrol                                                                        | C_{16}H_{10}O_{4} | Coumestans      | 297.048       |
| 21  | 11.293   | 352.0607   | 5’-O-Methyl(·)-epicatechin-5-O-sulfate                                                     | C_{24}H_{16}O_{5}S | Flavonoids      | 351.0607      |
| 22  | 11.448   | 624.2634   | Kanoside D                                                                                | C_{27}H_{44}O_{16} | Teprene glycoside | 623.2634      |
| 23  | 11.476   | 578.2573   | Withaperuvins H                                                                           | C_{30}H_{42}O_{8} | Withanolide     | 577.2573      |
| 24  | 11.515   | 400.2746   | Torosaflavone A                                                                           | C_{31}H_{20}O_{8} | Flavonoids      | 399.116       |
| 25  | 11.52    | 468.1045   | Gyrophoric acid                                                                            | C_{28}H_{28}O_{10} | Phytol          | 467.1045      |
| 26  | 11.561   | 330.241    | 5,8,12-trihydroxy-9-octadecenoic acid                                                     | C_{24}H_{14}O_{5} | Fatty acids      | 329.241       |
| 27  | 11.63    | 352.0947   | Psoralidin oxide                                                                          | C_{20}H_{16}O_{6} | Coumestans      | 351.0947      |
| 28  | 11.787   | 314.0793   | Luteolin 5,3’-dimethyl ether                                                               | C_{17}H_{14}O_{6} | Flavonoids      | 313.0793      |
| 29  | 12.099   | 256.0738   | 6-Demethylnigfuran                                                                         | C_{22}H_{22}O_{4} | Isolavonoid     | 255.0738      |
| 30  | 12.141   | 562.2625   | 19-Hydroxychromyzzanol 19-glucoside                                                       | C_{28}H_{42}O_{13} | Glicosides      | 561.2625      |
| 31  | 12.638   | 354.1101   | 2,3-Dehydrokrivotone                                                                       | C_{20}H_{14}O_{4} | Flavonone       | 353.1101      |
| 32  | 13.249   | 220.0737   | Polygenolide                                                                               | C_{22}H_{12}O_{4} | Coumarins       | 219.0737      |
| 33  | 13.368   | 322.1208   | 5,7-Dihydroxy-8-prenylflavone                                                             | C_{20}H_{16}O_{4} | Flavone         | 321.1208      |
| 34  | 13.375   | 368.1228   | Aurumilone                                                                                 | C_{21}H_{20}O_{6} | Flavonoe        | 367.1228      |
| 35  | 13.512   | 438.1681   | Morusagin L                                                                                | C_{25}H_{20}O_{7} | Flavones        | 437.1681      |
| 36  | 13.572   | 676.2315   | Arotin D                                                                                   | C_{26}H_{36}O_{10} | Chalcones       | 675.2315      |
| 37  | 13.573   | 452.11     | Cinchonain Ib                                                                              | C_{24}H_{20}O_{9} | Phytol          | 451.11        |

(Continued)
TABLE 5 | Enzyme inhibition effects of C. burhia aerial and root extracts.

| Extracts | AChE (mg GALAE/g extract) | BChE (mg GALAE/g extract) | Tyrosinase (mg KAE/g extract) | Amylase (mmol ACAE/g extract) | Glucosidase (mmol ACAE/g extract) |
|-----------|---------------------------|---------------------------|------------------------------|-----------------------------|-----------------------------|
| ChA-M     | 3.79 ± 0.27               | 2.93 ± 0.07               | 131.72 ± 0.52                | 0.63 ± 0.03                  | 1.86 ± 0.04                 |
| ChA-D     | 4.01 ± 0.41               | 4.28 ± 0.19               | 124.95 ± 0.35                | 0.67 ± 0.02                  | 1.92 ± 0.01                 |
| ChB-R     | 3.29 ± 0.34               | 3.37 ± 0.12               | 128.51 ± 1.35                | 0.60 ± 0.01                  | 1.89 ± 0.01                 |
| ChB-D     | 2.07 ± 0.16               | 2.32 ± 0.24               | 120.76 ± 0.40                | 0.70 ± 0.03                  | na                         |

GALAE, galatamine equivalent; KAE, kojic acid equivalent; ACAE, acarbose equivalent; na, not active. All values expressed are means ± S.D. of three parallel measurements.

To the World Health Organization, approximately 422 million individuals worldwide have been diagnosed with diabetes. Although synthetic medications have advanced, the number of people with diabetes continues to rise at an alarming rate. Several medicinal herbs, including curcumin, have been demonstrated to be beneficial in the diabetes (Choudhury et al., 2018; Obih et al., 2019). The alpha-amylyase and alpha-glucosidase inhibitors acarbose, miglitol, and viglibose have been established. Acarbose is derived from plants. Bloating, flatulence, and other gastrointestinal discomforts have been linked to an excess of alpha-amylase and alpha-glucosidase enzymes. The current investigations have revealed (Table 5) that C. burhia extracts a mild inhibitor of alpha-glucosidase and alpha-amylyase enzymes. The DCM root extract displayed the highest inhibitory potential against alpha-amylyase (0.70 mmol ACAE/g extracts) while DCM aerial extract presented maximum potential against alpha-glucosidase (1.92 mmol ACAE/g extracts). The alpha-amylyase inhibition results of C. burhia extracts were ordered as follows: Chb-R > Chb-D > Chb-A > Chb-R-M.

Cytotoxic activity

Two breast cancer cell lines, MCF-7 and MDA-MD-231, were tested for cytotoxicity of C. burhia extracts, as shown in Table 6. The results show that none of the extracts presented significant toxicity to the breast cell line used in the study. For MCF-7 and MDA-MD-231 cell lines, the Chb-A-M extract was found to be the most effective, with a percentage viability of 74.29 and 70%, respectively. Likewise, the Chb-R-M extract was also found to be considerably active against the MDA-MB-231 cell line, likewise, the Chb-D extract was also active against this cell line. The Chb-D extract was less toxic to either of the cell lines that were tested. In-vivo toxicity studies are...
TABLE 6  Cytotoxicity of *C. burhia* samples against breast cell lines.

| Extracts | % Viability (200 µg/mL) |
|----------|------------------------|
|          | MCF-7                  | MDA-MB-231               |
| ChA-M    | 74.29                  | 70.56                    |
| ChA-D    | 4.0297                 | 61.06                    |
| ChR-M    | 23.98                  | 84.04                    |
| ChR-D    | 14.546                 | 2.69                     |

ChA-M, *C. burhia* aerial methanol; ChA-D, *C. burhia* aerial DCM; ChR-M, *C. burhia* root methanol; ChR-D, *C. burhia* root DCM. Data from three repetitions, with mean ± standard deviation.

Recommended following this preliminary toxicity testing of the plant extract studied.

Principal component analysis

Data from multiple tests can be analyzed using PCA. To accomplish this, we used PCA to analyze the tested extracts. Correlation, clustering, and PCA were used to show how aerial and root extracts interacted with the biological assays. The results are summarized in Figure 2. Three dimensions summarizing, respectively, 50.6, 32.3, and 11.1% of the biological activities variability were obtained (Figure 2A1). It was noted that the two principal components were built by PCA, explaining 88.9% of the total variability, with dimension 1 (56.6%) and dimension 2 (32.3%) (Figure 2A2). Moreover, it was seen that the variables DPPH, ABTS, CUPRAC, tyrosinase, glucosidase, and AChE were strongly associated with the origination of axis 1 (56.6%), whereas, the variables inclusive of amylase, phosphomolybdenum, and BChE were strongly contributed to the formation of axis 2 (32.3%). The TPC was noted to be highly positive co-related with the CUPRAC, while a positive moderate co-relation was noted for the DPPH, and ABTS activities, whereas, a weak positive relationship was observed for the tyrosinase and glucosidase. Likewise, a moderate to weak negative correlation was observed among TPC and FRAP, PPBD, MCA, AChE, and BChE, while a strong negative co-relation occurred for the TPC and amylase. Similarly, the TFC presented a considerable positive relationship for CUPRAC, DPPH, ABTS, moderate to weak positive correlation for the tyrosinase, glucosidase, and FRAP, and a weak relationship for the PPBD, MCA, and amylase. These results are further verified from the heatmap.

Conclusion

The specific phytochemical and biological composition of several extracts of the *C. burhia* plant has emphasized the...
possible consequences of these extracts. Secondary metabolites in the phenolic, flavonoid, and glycoside classes were identified through HPLC-PDA and UHPLC-MS analysis. It was found that the most polar solvent extracts had the highest bioactive content. All of the tested extracts had varying antioxidant and enzyme-inhibiting potential. In addition, statistical studies confirm the link between the contents and the apparent biological activities. C. burhia plant extracts can be used as a natural source of bioactive compounds, according to the findings of this comprehensive report. However, more exploration is required for better insight in terms of isolation and characterization studies.

Data availability statement

The original contributions presented in this study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

SA, HS, and UK: writing and editing. MF, IP, NAK, KAM, and MH: data curation. KAI, FA, MS, ML, and NAH: supervision. All authors contributed to the article and approved the submitted version.

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Supplementary material

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Conflict of interest

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