Phytochemical Screening by HPLC and FTIR Spectroscopy of Glucokinin Isolated from Methanol Extract of Bauhinia Variegata

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Abstract

The isolation of active plants compounds lead to discover a several drugs derived directly from plants. In the current study, the Glucokinin was isolated and purified from the methanol leave extract of medicinal plant Bauhinia variegate, the attempts to identified and characterized it's functional groups by the HPLC and FT-IR spectroscopy was worked in this study. By using FT-IR spectroscopic the Glucokinin shown different functional groups. The FTIR spectrum of the purified material, Glucokinin from the methanol leaf extract of B. variegata indicate the presence of different functional groups. The peak near 1650 cm-1 is the amide I band.

Keywords: *Bauhinia variegate, Glucokinin, HPLC, FT-IR, medicinal plant.*

Introduction

Medicinal plants have been used for thousands of years in folk medicines in Asian and African populations and many plants are consumed for their health benefits in developed nations[1]. It is estimated that 70–95% of the population in developing countries continues to use traditional medicines[2]. Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity[3]. Plant polyphenols act as strong antioxidants and they protect cell constituents against oxidative damage, thus averting the deleterious effects on nucleic acids, proteins and lipids in cells[4]. The flavonoids, which are the largest and most studied polyphenols, are gaining interest as antioxidants because of their high capacity to scavenge free radicals. Flavonoids prevent hydroxy radical induced damage 10 by donating an electron to neutralize the species[5]. The isolation of plant active compounds not a new trend but begun before two century when the painkilling drugs were discover like morphine from *Papaver somniferum* L., digitoxin, the cardic glycoside, isolated from *Digitalis purpurea* and a lot other natural compounds that some of them still in use[6]. These compounds play strong role in development of therapeutics treatments[7]. *Bauhinia variegata* L which commonly known as mountain ebony, orchid-tree, poor-man's orchid, camel's foot and Napoleon's hat, belongs to the family Leguminosae[8]. It was planted in garden, park and roadsides as ornamental plant in many warm temperate and subtropical regions. It was native to Southeast Asia and grows in tropical and subtropical climate[9]. All parts of the plant (leaves, flower buds, flower, stem, stem bark, seeds and roots) were used in traditional medicine. It was traditionally used in the treatment of bronchitis, leprosy, tumors, astringent, tonic, anthelmintic, antidiabetic, laxative and for piles, used in the treatment of worm infestations, diarrhea and piles[10]. The phytochemical screening revealed that *B. variegata* contained terpenoids,

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flavonoids, tannins, saponins, reducing sugars, steroids and cardiac glycosides[11].

Pharmacological studies showed that *B. variegate* exerted anticancer, antioxidant, hypolipidemic, antimicrobial, anti-inflammatory, nephroprotective, hepatoprotective, antiulcer, immunomodulating, molluscicidal and wound healing effects[12]. A wide range of chemical compounds isolated so far from the plant are β-sitosterol, kaempferol-3-glucoside, tannins, carbohydrates, amides, reducing sugars, vitamin C, crude protein, fibers, calcium, phosphorus, quercetin, rutin, quercitrin, apigenin, apigenin- 7-O-glucoside, heptatriacontan-12, 13diol and dotetracontan-15-en-9-ol[13]. In addition, the presence of insulin-like molecules was demonstrated in the leaves of *B. variegata* where a protein was found that has a partial amino acid sequence identical to that of bovine insulin[14]. The activity of this insulin-like protein on serum glucose levels of four-week-old Swiss albino (CF1) diabetic mice was similar to that of commercial swine insulin used as control[11]. This study aim to identify the functional groups of Glucokinin that purified from the leave methanol extract of *B. variegate* by using HPLC and FT-IR techniques.

Materials and Method

Plant Preparation: The fresh leaves of *B. variegata* were collected from the gardens of Baghdad University, then washed well with tap water. The clean leaves then dried under shad, after that the dried leaves placed in oven for couples of hours. The dried leaves grinded by electric blender.

Plant Extraction: Fifty grams from the powdered leaves of *B. variegate* were taken and placed in 350 ml from %70 methanol for one week under room temperature and shaken between time to time. After that, the residues were taken off by filter paper, then the methanol was removed by using the rotary evaporator, in room temperature, until dryness.

Partial separation by column chromatography: Partial separation for flavonoids is done by using open glass column (2×17) cm which was filled by Sephadex LH20 (prepared by weighing 10g from powder Sephadex LH20 and dissolving it in 70% methanol and waiting until activated, approximately half an hour). This material separate the plant chemical components according to their molecular weight. Two gram from plant crude extract dissolved in 3 ml from 70% methanol, then added to the sephadex column, the elution solvent was 70% methanol also. When the chromatography was running 35 tube were collected with 5 ml from solvent in each one of tubes. All fractions were tested for $FeCl_3$ 1% solution as colorimetric test for polyphenols identification. Only the positive results elution's are collected.

Final purification by column chromatography: The same glass column (2×17) was used with new sephadex LH 20, the positive tubes that were collect from the first column were concentration by oven in 40-60 Ċ then were added to the second column, the elution solvent was methanol 70%. Twelve tubes were collected and the $FeCl_3$ 1% was used to test all the tubes, only the dark green tubes were taken.

Detection of phytochemical components: To detection of the essential phytochemical components the standard protocols were used which involved the basic colorimetric method that established to detect the presence of the major phytochemicals in extract as, terpenes and steroids tannins phenols saponins resins alkaloids and flavonoids[15, 16].

High performance liquid chromatography (HPLC): The HPLC for the methanolic extract of *B. vargiata* and the Glucokinin were carried out by using the following conditions According to[17].

Mobile Phase	**Acetonitrile : D.W (80 :20)**
Column	C 18 – ODS (25 cm * 4.6 mm)
Detector	UV – 280 nm
Flow rate	1 ml/min

Fourier transform infrared FT-IR spectroscopic analysis: Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined [18]. Five milligram from the isolated material mixed with 100 mg KBr pellet then loaded in in FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan), with a Scan range from 600 to 4000 cm^{-1}.

Results and Discussion

In this research, Glucokinin which isolated from the methanolic extract of *B. variegate* was screening for its properties and its chemical structure by using

different method such colorimetric test, HPLC and FT-IR techniques. The data of the colorimetric test for the crude methanol extract of *B. variegata* and the pure Glucokinin were shown in table (1) where observed that the crude extract have a positive result for all of the tannins, phenols, saponins, alkaloids, resins, flavonoids, terpenes and steroids and this agree with previous study that support the presence of various phytochemical compounds flavonoids, alkaloids, saponins, sterols, tannins and other components in the methanolic extract of *B. variegata* [19,20,21]. While the phenols and flavonoids were positive for the results of colorimetric Glucokinin tests.

The results that obtained from the HPLC were shown in Figure 1, 2and 3. In Table (2) shown that the partial purification of methanol extract of *B. variegata* have more than one compound with different retention times and one of these compound is similar in retention time of the insulin (3.464) which used as standard, the retention time of insulin shown in table (4). While table (3) elucidate the HPLC for the pure sample, Glucokinin, wherein one peak can be observed that and it's have the same retention time (3.448) with the retention time of insulin (3.464). This finding agrees with previous study that suggests the presence of insulin like protein (Glucokinin) in this plant[11].

Table 1: detection of some phytochemicals in crude methanol extract of Bauhinia variegata compare to Glucokinin

Phytochemicals	Crude extract	Glucokinin
Tannins	+	-
Phenols	+	+
Saponins	+	-
Alkaloids	+	-
Resins	+	-
Flavonoids	+	+
Terpenes	+	-
Steroids	+	-

Table 2: HPLC for partial purification of methanol.

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.752	397.524	34.283	4.0
2	3.448	8495.209	546.300	86.3
3	3.952	647.612	61.651	6.6
4	4.596	190.111	21.147	1.9
5	8.272	115.417	7.697	1.2
	Total	9845.873	671.079	100.0

Table 3: HPLC for Glucokinin extract of B. variegate

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	3.448	1941.018	222.024	100.0
	Total	1941.018	222.024	100.0

Table 4: HPLC for insulin

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	3.464	1733.879	251.386	100.0
	Total	1733.879	251.386	100.0

The FTIR spectrum of the purified material, Glucokinin from the methanol leaf extract of *B. variegata* indicate the presence of different functional groups. The results shown in Fig. (1) and Table (5). The peak near 1650 cm^{-1} is the amide I band. It results from the C=O stretching of the peptide bond. Similarly, the peaks near

1540 cm-1 (N-H bending/C-N stretching) and 1240 cm-1 (C-N stretching/N-H bending) are called the amide II band and amide III band, respectively. Also the peak near 3300 cm-1 is thought to be N-H bending and the peak near 1400 cm-1 to result from protein side-chain COO-. The finding of protein side chain consider logical result as in previous study the amino acids sequence of Glucokinin found to be partially similar to the sequence of bovine insulin[14]. As the absorption peak position and shape of the amide I band differ according to the secondary structure, peak analysis can yield information on the secondary structure.

Table 5: FTIR spectral peak values and functional groups obtained from the isolated material

Peak Values	Functional Groups
3383	OH group
3300	N-H bending
2929	C-H stretching
2858	CH3
1724	C=O carbonyl group
1651	C=O stretching of the peptide bond, amide I
1540	N-H bending/C-N stretching, amide II band
1361	protein side-chain COO-.
1240	C-N stretching/N-H bending, amide III
1062	C-O group

Conclusion

This compound play an important role in the medical value of *B. variegata* as anti-diabetic plant. The success of isolation and purification of Glucokinin from plants, which consider as less toxic, lower cost and less cross reactive effects supply the medical fields with alternative source of human and animal insulin.

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