Section A-Research paper

Phytochemical and Physiochemical evaluation of the medicinal herb, Citrullus colocynthis and Trigonella foenum graceum for *in vivo* anti-diabetic activity

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Abstract:

Citrullus colocynthis and Trigonella foenum graceum are known in Ayurvedic medicine for their hypoglycemic and anti-diabetic properties. Traditional medicine involves the use of various plant extracts for phytochemical bioactive ingredients, which provide health benefits at an affordable price. Secondary metabolites are responsible for the medicinal activity of these plants. **Method:** Pharmacognosis study, physiochemical and qualitative phytochemical analysis of these plants, moisture content, pH, alcohol soluble extract, water soluble extract, total ash, acid soluble ash, water soluble ash, amino acids, Different test methods were used to find out. Phenols, tannins, flavonoids, proteins, sugar lowers, carbohydrates, lipids, saponins, alkaloids, glycosides, steroids and TLC. **Result:** The test confirms the presence of various parameters in physiochemicals and phytochemicals, mainly alkaloids, glycosides, flavonoids, carbohydrates, amino acids, proteins, bitter principles and saponins. **Conclusion:** The result shows the presence of phytochemical properties of these plants are bio-active component and responsible for these hypoglycemic and anti-diabetic effects in diabetic mice.

Keywords: Diabetes, hypoglycemic, phytochemical, Citrullus colocynthis and Trigonella foenum graceum

Introduction

Herbal medicines have been used to treat various ailments and a large part of the world's population still relies entirely on traditional medicine. Many medicinal plants and their formulations are being used in Ayurvedic medicine systems as well as in ethnic medicine to treat diabetes [1]. From ethno-botanical data, more than 1,200 species of plants have been tested for anti-diabetic activity based on ethno-drug use, and approximately 800 plants have been reported to have diabetes potential [2, 3]. Many plants have been used as nutritional supplements and have also been used in the treatment of numerous ailments even without any knowledge of their proper function and ingredients, mainly compared to their synthetic hypoglycemic agents. Due to

the low side effects and their safety, effectiveness and availability. There are about 200 pure compounds from plant sources that lower blood glucose levels. The compounds are mainly alkaloids, antherecene derivatives, glycosides, flavonoids, bitter principle, coumarins, and saponin. The families of plants with the most powerful hypoglycemic effects include leguminoseae, lamiaceae, liliaceae, cucurbitaceae, asteraceae, moraceae, rosaceae, euphorbiaceae and araliaceae. [4] Although hundreds of conventional plants have been identified with physicochemical and phytochemical compounds, only a small number of them have been scientifically tested for their efficacy. Therefore, the present study was carried out with aqueous and alcoholic extracts of Citrullus colocynthis and Trigonella foenum graceum, respectively, which have been reported to contain various compounds [5, 6, 7] that are helpful in the treatment of diabetes in humans.

MATERIALS & METHODS

Material

Test Sample:

Citrullus colocynthis and *Trigonella foenum graceum* were collected from the herbarium of Bilwal medchem and research laboratory pvt. ltd., Jaipur. Roots of *Citrullus colocynthis* and seeds of *Trigonella foenum graceum* were used to carry out the experimental work.

Method

Pharmacognosy Study

The collected sample was studied organoleptically with naked eye &magnifying lens, with the help of Pharmacognostical procedure i.e. Taste, Odour and colour findings were recorded.

Powder microscopy

Powder microscopic inspection of medicinal plant materials is indispensable for the identification of broken or powdered materials; the specimen has to be treated with chemical reagents and observed under the microscope. Chemical reagents used for staining of the powder samples were as follows Safranin, Dilute Ferric chloride, Eosine, Methylene blue, HCl, Phlorogucinol, Iodine solution.

Physiochemical Analysis

Determination of Moisture Content, pH, Alcohol Soluble Extractive, Water Soluble Extractive, Total Ash, Acid Insoluble Ash, Water–soluble Ash was performed of Citrullus colocynthis and Trigonella foenum graceum.

Phytochemical Analysis

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Qualitative analysis of extracts was evaluated for general phytochemical profile like amino acids, phenols, tannins, flavonoids, proteins, reducing sugar, carbohydrates, lipids, saponins, alkaloids, glycosides, and steroids.

Thin Layer Chromatography

Thin layer Chromatography is a tool for separation and identification of chemical constituent. Thin-layer chromatography is a technique in which a solute undergoes distribution between two

phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Procedure: T.L.C. plate coated with 0.25 mm layer of silica gel 60 F_{254} with fluorescent indicator was used. (Each plate dimension is 10 cm long and 2 cm width)**.** Plates were dried in hot oven at 105^0 C for one and half hour, mobile phase - Toluene: Ethyl Acetate: Formic Acid (6: 3: 1). For test sample- 4 gm powdered drugs were extracted with 100 ml of ethanol (90 percent) in a Soxhlet apparatus consecutively three times. Extract was filtered and concentrated to 10 ml. Samples were applied with the help of capillary 1(one) cm above the base of T.L.C. plate. Then it was dipped in mobile solution. T.L.C. plate was removed from the mobile solution immediately after the spot reached the 1(one) cm below the top of the T.L.C. plate. p-anisaldehydesulphuric acid was used as visualization.

Estimation of Total steroids content and saponin % was also performed

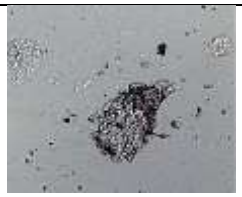
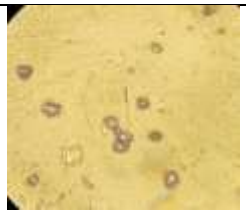
Result

Pharmacognostic analysis of *Citrullus colocynthis & Trigonella foenum graceum*

Table 1: Macroscopic study of *Citrullus colocynthis & Trigonella foenum graceum*

S. No	Tests	*Citrullus colocynthis*	*Trigonella foenum graceum*
1	**Taste**	Bitter	Bitter
2	**Odour**	Characteristics	Characteristics
3	**Colour**	Light Brown	Light Brown

Figure 1: Powder microscopy

Citrullus colocynthis		
		
Pitted vessels	Trichomes	Cork cells
		
Fibers	Starch Grain	Cork Cells
Trigonella foenum graceum		

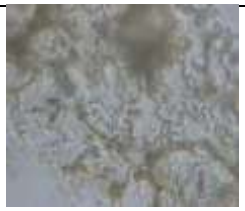
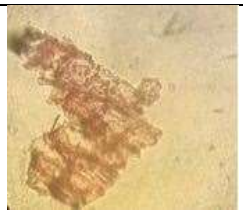
		
Stomata	Trichomes	Cork cells
		
Fibers	Vein islet	Spiral vessel

Table 2: Physiochemical Analysis

S. No.	Test	*Citrullus colocynthis*	*Trigonella foenum graceum*
1.	Moisture content (%)	6.47	4.6
2.	pH value	5.1	5.3
3.	Aqueous soluble extract (%)	18.74	7.3
4.	Alcohol soluble extract (%)	12.57	6.1
5.	Petroleum ether soluble extract (%)	1.10	5.9
6.	Total ash (%)	12.98	6.6
7.	Acid insoluble ash (%)	5.48	1.2
8.	Water soluble ash (%)	7.61	2.1

Table 3: Phytochemical Analysis of extract *Citrullus colocynthis* and *Trigonella foenum graceum*

S. No.	Name of test	*Citrullus colocynthis*		*Trigonella foenum graceum*	
		Aqueous extract	Alcohol extract	Aqueous extract	Alcohol extract
Determination of Carbohydrate					
A.	Molisch test	+ ve	+ve	- ve	+ve
B.	Benedict test	+ ve	- ve	+ ve	- ve
C.	Barfoed's test	- ve	- ve	- ve	- ve
D.	Fehling test	+ ve	+ ve	-ve	+ ve
Determination of Alkaloids					
A.	Dragondrof test	+ ve	+ ve	-ve	+ ve
B.	Wagner's test	- ve	+ ve	- ve	+ ve
C.	Hager's test	+ ve	- ve	+ ve	- ve

Determination of Amino acids					
A.	Ninhydrine test	+ ve	+ ve	+ve	+ ve
Determination of Proteins					
A.	Biuret test	+ ve	- ve	+ ve	- ve
B.	Xanthoprotic test	+ ve	+ ve	- ve	+ ve
C.	Millon's test	- ve	- ve	- ve	+ve
Determination of Saponin					
A.	Foam test	+ ve	- ve	+ve	- ve
Determination of Glycosides					
A.	Borntragor's test	+ ve	- ve	+ve	- ve
Determination of Phenolic compound					
A.	Phenolic test	- ve	- ve	- ve	- ve
Determination of Steroids					
A.	Salkowaski reaction	+ ve	- ve	+ve	- ve
Determination of Tannin					
A.	$FeCl_3$test	- ve	- ve	- ve	- ve
B.	Lead acetate test	+ ve	+ ve	+ ve	+ ve
C.	Potassium dichromate test	- ve	- ve	- ve	- ve

Figure 2: Estimation of Thin Layer Chromatography of *Citrullus colocynthis* and *Trigonella foenum graceum*

Citrullus colocynthis		***Trigonella foenum graceum***	
			
UV	**p-anisaldehydesulphuric acid**	**UV**	**anisaldehydesulphuric acid**

R_f Value: 0.16, 0.19, 0.24, 0.36, 0.45	R_f Value: 0.16, 0.19, 0.24, 0.36, 0.45	R_f Value: 0.15, 0.17, 0.22, 0.34	R_f Value: 0.12, 0.38, 0.46, 0.49 0.51, 0.58, 0.62, 0.86

Table 4: Estimation of Total steroids content

S. No	Name of Sample	% of Steroids
1	***Citrullus colocynthis***	1.24%
2	***Trigonella foenum graceum***	1.26 %

Table 5: Estimation of Saponins

S. No	Name of Sample	% of Saponins
1	***Citrullus colocynthis***	2.41%
2	***Trigonella foenum graceum***	3.11%

Discussion

Medicinal plants are extensively used for management of health since a long time and are becoming popular not only in village folk but also in urban people. The medicinal value of plants mainly depends upon their active ingredients or the bioactive substances such as alkaloids, flavanoid, saponins, glycosides and other related active metabolites which are extensively used in the drug and pharmaceutical industry. Thus the preliminary phytochemical tests are helpful in finding chemical constituents of a plant material to which the biological effect obtained could be correlated effectively [8, 9].

Phytochemical and physiochemical analysis of the Citrullus colocynthis and Trigonella foenum graceum was carried out using standard procedure in the present study before conduct of the experiment. In the present study, the alcoholic and aqueous extract of both Citrullus colocynthis and Trigonella foenum graceum were found positive for alkoloid, flavonoid, amino acids, carbohydrates, proteins, saponin and glycoside derivatives. Several earlier workers have subjected Trigonella foenum graecum for their phytochemical analysis [10-15] and have observed presence of alkaloid, saponin, flavonoids, bitter principles, coumarins, anthracence derivatives and glycosides similar to the results of the present study. [10] While subjecting petroleum extract, ethalonolic extract and aqueous extract for phytochemical analysis obtained steroids in trigonella seeds only in petroleum extract and absent in aqueous and alcoholic extracts. [11] On phytochemical analysis observed that the steroidal saponins were in large quantity in trigonella seed extract [15] and subjected crude ethanol extract of T. foenum graecum seeds for phytochemical analysis to check the presence of alkaloid, steroid, flavonoid, carbohydrate, glycoside and glucosides in it and observed the presence of alkaloid, steroid and carbohydrate but no flavonoid, glycoside and glucosides in the crude seed extract, [13] showed the presence of alkaloid, flavonoids, amino acid, tannins, protein, starch, mucilage and saponins in the methonolic and aqueous extracts of Trigonella. Amino acids like isoleucine, 4-

hydroxyisoleucine, histidine, leucine, lysine, L-tryptophan, argenine; saponins like graecunins, fenugrin B, fenugreekine, trigofoenosides A-G; Steroidal sapinogens like yamogenin, diosgenin, smilagenin, sarsasapogenin, tigogenin, neotigogenin, gitogenin, neogitogenin, yuccagenin, saponaretin; Fibers like gum, neutral detergent fiber and others like coumarin, lipids, vitamins, minerals, 28 per cent mucilage; 22 per cent proteins; 5 per cent of a stronger-swelling, bitter fixed oil components were reported [15] . It also observed presence of glucosides, phenol, flavonols, amino acid, alkaloides, steroids, tannin, polysaccharide, pectin and hemicelluloses, fats volatile oil in the ethanolic extract of fenugreek. [14] It has been indicated that fenugreek (Trigonella foenum graecum) helps in balancing cholesterol, lowering sugar level, curing skin inflammation (wounds, rashes, boils), treating arthritis, asthma, sore throat, due to the phytoconstituents such as flavonoids, alkaloids, terpenoids, steroids, saponins, anthocyanin, tannin etc. Glucose-lowering and antidiabetic effects of fenugreek have been attributed to the galactomannan rich soluble fiber fraction of fenugreek. Insulinotrophic and antidiabetic properties also have been associated with the amino acid 4- hydroxyisoleucine that occurs in fenugreek at a concentration of about 0.55 per cent. In vitro studies have indicated that this amino acid causes direct pancreatic β-cell stimulation. The alcoholic leaf extract of Citrullus colocynthis was reported to possess tannins, saponins, alkaloids, flavonoids, carbohydrates, triterpenoids, glycosides etc on phytochemical analysis [16, 17]. They have reported varying effects of the extract and attributed to antioxidant activity and alkaloids to be responsible for antidiabetic property [18, 19].

Conclusion

Plants that are rich in secondary metabolites, called medicinal plants, are widely used in traditional medicine to fight and treat various diseases. Many of the plants studied have been used in medicine since the time of Ayurveda, an ancient system of Indian medicine. The various biochemical ingredients in the extract of the leaves and seeds of medicinal plants include anthocyanins, steroids, terpenoids, coumarins, fatty acids, tannins, saponins, leukoanthocyanins and immunodeficiencies. The anti-inflammatory, antispasmodic, antianalgesic and diuretic effects can be attributed to the high levels of steroids, tannins, terpenoids, saponins and glycosides in medicinal plants. It has been used to treat aphrodisiac, neuroprotective, liver tonic, astringent, and bronchitis, asthma, ulcers, weakness, insomnia, geriatric dementia, and diabetes. While medicinal plants have been used successfully in Ayurvedic medicine for centuries, more clinical trials should be conducted based on the presence of phytochemicals in leaf extract for secondary metabolites to help in its therapeutic use. Alcohol and aqueous extracts Trigonella foenum graecum and Citrullus colocynthis were found to be positive for saponins, alkaloids, bitter principles, carbohydrates, proteins, amino acids, tannins and glycosides. Rf value indicates the presence of active ingredients in Trigonella foenum graecum and Citrullus colocynthis.

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