PHYTOCHEMICAL ANALYSIS of CANNABIS SATIVA IN DISTRICT KECH, BALOCHISTAN, PAKISTAN

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Abstract

Objective: The aim of this work is to identify and quantify the phytochemical chemical constituent present in cannabis Sativa, which is an important herbal plant and generally used for medicinal and recreational purposes

Methodology: The leaves, stem, and roots of the selected plant were collected, dried, and crushed into powder form, and extract for phytochemical analysis

Results: The range of alkaloids in leaves was 5.82%, the stem was 4.20%, and the roots were 2.25%. The value of saponin in leaves, stems, and roots was 1.62%, 1.55%, and 1.52%. The content of tannin in leaves was 3.00%, the stem was 3.22%, and the roots were 3.12%. The value of phenols in leaves, stem, and roots were 1.10%, 1.05%, and 1.01%

Keywords: Herbal plant, Cannabis sativa, Phytochemical

Introduction

Cannabis sativa is a type of an important herbal plant, grown all over the world, belonging to the family Cannabaceae and has been used in different regions of the world as a source of oil and for medicinal purposes [1] Cannabis sativa contains a highly complex mixture of compounds, [2] and up to date, 568 unique compounds are identified in the cannabis [3]. The most important classes are cannabinoids, terpenoids, nitrogenous compounds, non-cannabinoid, phenols, flavonoids, and steroids. Among these compounds, tetrahydrocannabinol (THC), cannabinol (CBN), and cannabinodiol (CBND) are known to be psychoactive [3]. There are three species of Cannabis plant namely C. Sativa, C. indica and C. Ruderalis, and they are found in Russia, China, India, Iran, and Pakistan[4]. C. Sativa and C. Indica are often grown all over the world while in Pakistan, they are naturally grown as the wild form in mountainous and rural areas[4]. The wild-type plant of C. ruderalis shows natural distribution in the low land regions has a low concentration of cannabinoid and non-cannabinoid compounds. However, the C. indica and C. Sativa types that are commonly found in the Hindukush, North mountainous, and Karakoram regions have a higher concentration of cannabinoid compounds [5]. C. Sativa is naturally grown in different parts of the Punjab province like in Sialkot, Layyah, Mianwali, Muzaffargarh, D. G khan, and in the hilly area of Islamabad, in Balochistan cannabis grown mountains areas of Quetta, Kalat and Mekran [6]. As a crop, it is grown at a very low scale in different areas of Pakistan and is commonly known as bhang in the local language [5]. In the modern system of the breeding and cultivation of the plant for recreational and medicinal purposes, cannabis can propagate by cloning, using cuttings of a so-called ‘mother plant. In this way, female plants are used for this cloning purposes, so, they yield a higher amount of psychoactive compounds than the male plant [7]. Medicinal plant extracts contain various types of bioactive compounds known as phytochemicals. These phytochemicals can be used in treatment as anticancer, antimicrobic, antioxidant, and anti-inflammatory agents. Recent studies show that these phytochemicals are safe, broadly effective, and have a fewer adverse effects[8]. Herbal plant medicines are likely to be unhygienic with heavy metals. In trace amounts some

heavy metals are essential for the human body however, they may be toxic if present in a higher concentration. They have the ability to bioaccumulation and disturb the functions of key organs and glands in the human body such as the brain, kidney, and liver[8]. The history of the Cannabis sativa plant is very close to the beginning of human beings was known. Around 141 million people worldwide use cannabis Sativa.[9]. The rate of using cannabis is highest in developing countries due to poverty and the expensive price of medicine. Different parts of the cannabis plant, shoot, bark, and leaves are used to get relieve pain and help in the control of disease, since the first time it has been mentioned that the people of Africa and Asia have used Cannabis in different forms, usually smoking it and often mixing it with tobacco or with drinks or sweetmeat [10]. Chinese, Indian surgery, Egyptian, Hellenic medicine, and the Arab world used the Cannabis plant as a medicinal herb for various diseases, as on painkiller, and as an antiseptic applied externally to get relive from pain [11].

This study was, therefore, designed to investigate phytochemical screening, in three parts (leaves, stems, and roots) of the plant Cannabis sativa.

Materials and Methods

Sample Collection

Fresh whole plants of cannabis sativa were collected from different mountainous areas of (Buleda, Tump and Shapuk) in District Kech, Balochistan, Pakistan

Samples Preparation

The leaves, roots and stem of the cannabis plant were separated from the plants and then washed under running tap water to removed dust. The plant samples were dried for few days.

Preparation Powder of Cannabis Sativa

After drying, the selected parts (stems, leaves, roots) were crushed into powder using electric blender.

Preparation of plant extracts

10 g powdered of each parts of plant were dissolved separately in 100 ml of ethanol, petroleum ether, and distilled water separately and kept on shaking bath at room temperature for 24 hours and then filtered and centrifuged. The supernatant was kept in

oven at 40 C0 for four hours. The remaining solution was stored in refrigerator for further analysis

Qualitative Analysis of Phytochemical

Standard procedures were used for qualitative analysis of samples to find out presence of following phytochemical, carbohydrates, proteins, alkaloids, glycosides, saponins, tannin, phenols, terpenoids, steroids, and flavonoids[12],[13].

Carbohydrates (Molish's test)

2 ml samples a few drops of alcoholic, and α-naphthol, 1 ml of concentrated sulphuric was added sideways of the test tube. The violet colour shows the presence of Carbohydrates

Test for proteins (Biuret test)

Proteins test was finished with equivalent volumes of 1% sodium hydroxide and 1% copper sulfate. The purple showed the presence of proteins.

Test for alkaloids

In 1ml of the plant extract, 2ml. of Mayer's reagent (potassium mercuric iodide solution) was added. Presence of cloudy white precipitate showed the presence of alkaloids.

Test for glycosides (Keller- Killiani test)

1 mL sample, added 2 mL of glacial acetic acid, one drop of ferric chloride, and 1 mL of concentrated sulphuric acid was added. A violet ring indicated the presence of glycosides.

Test for Saponins (Foam test)

1ml of the extract was added with 20ml of distilled water. The solution was shaken for 10 to 20 min till the formation of foam. The presence of saponins in the extracts indicated the presence of foam in the extract.

Test for tannins

2 mL extract, 1% ferric chloride solution was added. Development of brownish-green or blue-black coloring showed the presence of tannins.

About 2 ml of the extract, 2 drops of sulphuric acid, and 5 drops of 5% hydrochloric acid were mixed together. Green color formation showed the presence of tannins.

Test for terpenoids (Salkowski test)

5 ml of each extract was mixed in 2ml of chloroform and concentrated sulphuric acid (3ml.) and it was carefully added to form a layer. A reddish brown precipitate of the boundary showed the presences of terpenoids

Test for steroids (Liebermann Buchard test)

The plant extract was dissolved in 2ml. of chloroform to which 10 drops of acetic acid and five drops of concentrated sulphuric acid were added and mixed. The change of red color through blue to green indicated the presence of steroids.

Test for Anthraquinones (Borntrager s test)

1 ml of the extract with 2 ml of chloroform shacked strongly and added an equal volume of 100% ammonia solution. Let the solution mix well so, the formation of pink, violet, or red color indicated the presence of anthraquinones.

Test for flavonoids

2 ml of extract were added with a couple of drops of 10% ferric chloride. The green shading shows the presence of flavonoids.

Quantitative Analysis of Phytochemical of Cannabis Sativa

The Quantitative analysis of alkaloids, saponins tannins, and phenols were carried out from standard methods [14].

Result

This result showed that proteins, alkaloids, saponins, tannins, and phenols, were present in three parts of the plant (leaves, stems, and roots). Steroids were present in water and ethanol extracts, while absent in ether extract as shown in (table,1,2,3). The Anthraquinones were present weakly in all extracts which showed only layers in the bottom of the test tube but did not show any kinds of colours. Flavonoids were present in water extract while absent in ethanol, and ether extracts in whole parts of the plant (leaves, stems, and roots). Carbohydrates and glycosides were absent in all three parts (leaves, stems, and roots) of plants, no reaction occurred with any kinds of reagents. Bauhinia Variegata showed the presence of seven [15] phytochemicals such as alkaloids, tannins, saponins, glycosides, flavonoids, phenols, and triterpenoids. The Quantitative study of phytochemicals were showed in (table 4). The value of alkaloids in leaves, stem .and roots were 5.82%, 4.20%, and 2.25%, The range of saponins in leaves, stem, and

roots were 1.62%, 1.55%, and 1.52% respectively. The value of tannin in leaves was 3.00%, stem was 3.2 %, and roots was 3.12%. The value of phenol in leaves, stems, and roots were 1.10%, 1.05%, and 1.01%. The present analysis shows that significant variation in the contents like alkaloids, flavonoids, phenol and saponin The present investigation shows that significant variation in the contents like alkaloids, flavonoids, phenol and carbohydrate when compared to below mentioned results. These variations are due to number of environmental factors such as climate, altitude, rainfall etc. as mentioned[16]. when compared to above mentioned results. These variations are due to number of environmental factors such as climate, altitude, rainfall etc. as mentioned [16].

Table.1 Qualitative analysis of Phytochemical of Cannabis Sativa (Leaves)

Phytochemical	Ethanol	Petroleum Ether	Aqueous
Carbohydrates	_	_	_
Proteins	+	+	+
Alkaloids	+	+	+
Glycosides	_	_	_
Saponins	+	+	+
Tannins	+	+	+
Phenol	+	+	+
Terpenoids	+	+	+
Steroids	+	_	+
Anthraquinones	+	+	+
Flavonoids	- _	_ -	+

Table .2 Qualitative analysis of Phytochemical of Cannabis Sativa (Stems)

Phytochemical	Ethanol	Petroleum Ether	Aqueous
Carbohydrates	_	_	_
Proteins	+	+	+
Alkaloids	+	+	+
Glycosides	_	_	_
Saponins	+	+	+
Tannins	+	+	+
Phenol	+	+	+
Terpenoids	+	+	+
Steroids	+	_	+
Anthraquinones	+	+	+
Flavonoids	-	-	+

Table.3 Qualitative analysis of Phytochemical of Cannabis Sativa (Roots)

Phytochemical	Ethanol	Petroleum Ether	Aqueous
Carbohydrates	_	_	_
Proteins	+	+	+
Alkaloids	+	+	+
Glycosides	_	_	_
Saponins	+	+	+
Tannins	+	+	+
Phenol	+	+	+
Terpenoids	+	+	+
Steroids	+	_	+
Anthraquinones	+	+	+
Flavonoids	-_	-	+

Table 4 Quantitative Analysis of Phytochemical Constituents of Cannabis Sativa plant

Parameters	Leaves%	Stems%	Roots%
Alkaloids	5.82	4.27	2.25
Saponin	1.62	1.55	1.52
Tannin	3.00	3.22	3.12
Phenol	1.10	1.05	1.01

Discussion

The analysis of phytochemicals revealed that alkaloids, tannins, saponins, and phenols were present at a high level in cannabis Sativa leaves, stems, and roots. The presence of alkaloids in leaves was 5.82% shows that the leaves could be utilized in the treatment of hypertension, agony, sedative, and expanding the activity of chemicals[17]. The presence of tannins in cannabis showed as antitumor, antibacterial, antidiabetic, and furthermore, defer HIV propagation Saponins, were additionally present in plants that have anti-microbial properties, safeguard against hypercholesterolemia and hyperglycemia.

Presences of phenols in cannabis showed that cannabis is used as painkilling, insecticidal, and antimicrobial [18]. further study shows that Phenols are reported antitumor agents and exhibit antiviral and antimicrobial activities [19] hypotensive effect [20] and antioxidant properties[21]. With the presence of specific phytochemicals, this plant is extremely helpful to plant for the therapy of the accompanying sicknesses hack, cerebral pain, aggravation, wound recuperating, expectorant, gastrointestinal issues, carminative, asthma, clean, antidiabetic, disease, weight reduction.

Conclusion

It is uncovered that the plant Cannabis Sativa is utilized for clinical, and receational purposes. These plants have therapeutic properties in view of their phytochemical parts. These phytochemicals show remedial, and loosening up consequences for humanity. From this ongoing review, we inferred that the leaves, stem, and roots separates showed high potential phytochemical properties. Concentrates of this plant are plentiful in alkaloids, proteins, saponins, and phenol constituents. The therapeutic properties of Cannabis Sativa might be because of the presence of the dynamic phytochemicals. Consequently, there is a need to investigate the pertinence of these plant assets which are wealthy in phytochemicals and may helpfully affect wellbeing.

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Conflicts of Interest

The authors declare no conflict of interest.

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