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VARIATION IN PRELIMINARY PHYTOCHEMICALS SCREENING OF *CANNABIS SATIVA* L. LEAF, STEM AND ROOT

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ABSTRACT: Qualitative phytochemical screening of *C. sativa* leaf, stem and root was studied. Four solvents *viz*; *n*-hexane, chloroform, alcohol and aqueous revealed the variation in phytochemicals presence and absence in all studied parts. Extracts of different solvents were subjected to qualitative phytochemical screening using a standard protocol. Results showed the maximum number of phytochemicals present in leaf followed by stem and root. They are as follows; steroids, fixed oil, resins, alkaloids, terpenoids, flavonoids, tannin, proteins, and amino acid, phenolics, glycosides, saponins. Variation in a number of phytochemicals also observed in different solvents extracts.

INTRODUCTION: Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, *etc. i.e.,* any part of the plant body may contain active components [1]. The quality and quantity of phytochemicals present in plant parts may differ from one part to another. There is lack of information on the distribution of the biological activity in different plant parts essentially related to the difference in the distribution of active compounds (or active principles) which are more frequent in some plant parts than in others [2].

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Successful determination of biologically active compound from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions include low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action and inability to cause the extract to complex or dissociate.

As the end product in extraction will contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the bioassay [3]. Screening of various natural organic compounds and identifying active agents is needed of the hour as due to successful prediction of lead molecules and drug-like properties at the onset of drug discovery will pay off later in drug development [4]. This, therefore, underscores the need to try as many solvents as possible in screening plant parts for phytochemicals. *Cannabis sativa* L. is one of the oldest dioecious plant known in medicine and one that has been most studied concerning its

phytochemistry [5]. Phytochemistry of *Cannabis sativa* belongs to different classes such as alkaloids, terpenoid, glycosides, steroids, phenolics, aliphatic compounds, hydrocarbons and polysaccharides. A detail of phytochemicals of this species is already documented [6].

MATERIALS AND METHODS:

Collection and Sample: Fresh parts of *Cannabis sativa* leaves, stem and roots were collected from the Aligarh. The samples were identified at Department of Botany, Aligarh Muslim University, Aligarh.

Extraction: The material was washed thoroughly 2-3 times with running tap water, and then air dried under shade after complete shade drying. The dried material was ground in mixer; the powder was kept in small plastic bags with paper labelling.

Assembly was arranged, and thimble was prepared. 10 gm of air-dried powdered drug was extracted with hexane for 3 days then extract solution was collected and concentrated under vacuum using rota-vapour. Then the plant material was again collected and air dried. When completely dried it was again packed back in the thimble. The same method was repeated for chloroform, alcohol, and water. Finally, the dried extracts were collected in pre-weighed glass vials and post-weight for each vial was taken. Extracts for leaf, stem, and roots of *C. sativa* were collected and finally, the percentage yield was calculated for all the extracts of all the parts [7].

Phytochemical Screening: The extract was tested for the presence of bioactive compounds by using the following standard methods [8-10].

Steroids: Crude extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing the crude extract with 2 ml of chloroform. Then 2 ml of each of concentrated H_2SO_4 and acetic acid was poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

Terpenoids: Crude extract was dissolved in 2 ml of chloroform and evaporated to dryness. To this, 2 ml of concentrated H_2SO_4 was added and heated for about 2 min. A grayish colour indicated the presence of terpenoids.

Fatty Acids: 0.5 ml of extract was mixed with 5 ml of ether. These extract was allowing it for evaporation on filter paper and dried the filter paper. The appearance of transparency on filter paper indicates the presence of fatty acids.

Tannins: 2 ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins.

Test for Phenolic Compounds: Formation of intense green, purple, blue or black colours with the addition of 1% ferric chloride solution to the extract.

Test for Alkaloids: 200 mg plant extract is dissolved in 10 ml methanol and then filtered. In 1 ml filtrate, 6 drops of Dragondroff's reagent is added. The appearance of orange precipitate indicates the presence of alkaloids.

Test for Flavonoids: 5 ml of dilute ammonia solution was added to the filtrate followed by concentrated sulphuric acid. A yellow colour observed indicates the presence of flavonoids.

Tests for Proteins and Amino Acids: Test solution with 2 ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid), white precipitate appears, which turns red upon gentle heating.

Test for Fats & Fixed Oils: Add a few drops of 0.5N of alcoholic potassium hydroxide to small quantities of various extracts along with a drop of Phenolphthalein separately and heat on a water bath for 1-2 h. The formation of soap or partial neutralization of alkali indicates the presence of Fixed oils and Fats.

Saponins: 5 ml of extract was mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 min. Formation of foam indicates the presence of saponins.

RESULTS AND DISCUSSION: Analysis of the plant extracts revealed the presence of phyto-chemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids. The phenolic compounds are one of the

largest and most ubiquitous groups of plant metabolites [11]. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, anti-inflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [12].

Results obtained for the qualitative screening of phytochemicals in *C. sativa* all parts are presented in **Table 1.** Leaf extract showed the presence of all the tested phytochemicals. They are steroids, fixed oil, resins, alkaloids, terpenoids, flavonoids, tannin, proteins, and amino acid, phenolics, glycosides, saponins, however, in stem extract saponins showed absence in aqueous extract while, in root extract fixed oil and saponins showed absence in their respective selected solvents. Water is a universal solvent, used to extract plant products with activity. Though traditional healers use water primarily plant extracts from organic solvents have been found to give more activity compared to water extract [13].

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF *C. SATIVA*

Extracts	Chemical constituents	Leaf	Stem	Root
n-Hexane	Steroids	++	+	+
	Fixed oil	+	+	-
Chloroform	Resins	++	++	++
	Alkaloids	++	+++	++
	Steroids	+	+	-
	Terpenoids	+++	+++	++
Alcohol	Resins	+++	+++	++
	Flavanoids	+++	++	+
	Tannin	++	++	++
	Phenolics	++	++	+
	Alkaloids	+	++	+
	Terpenoids	+	+++	++
	Proteins and amino acids	+	+	+
Aqueous	Tannins	+++	+++	+
	Flavanoids	++	++	++
	Phenolics	+++	++	+++
	Glycosides	++	+	+
	Saponins	+	-	-

Sterols and terpenes are organic compounds. The organic compounds are insoluble in water, which explains the absence of sterols and terpenes in the aqueous extract as shown in **Table 1**.

In root extract, steroids showed absence in *n*-hexane however present in chloroform extract. In the present study, phytochemicals showed variation in number from part to part which also supported by [7].

CONCLUSION: Phytochemicals found in extracts of *C. sativa* indicates their potential as a source of therapeutic constituents that may supply novel medicines. Further studies are therefore suggested to ascertain their various pharmacological activities such as antimicrobial, antispasmodic, analgesia and antihelminthic activities.

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