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ESTIMATION OF PHYTOCHEMICAL COMPOUNDS FROM FLOWERS OF SALVIA

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Abstract:

In this research paper, the primary and secondary metabolites present in the flower of Salvia divinorum have been studied. Primary metabolites such as total soluble sugar, carbohydrate, protein and phenol were quantitatively analyzed. Similarly, secondary metabolites like flavonoids phytosterols were also analyzed quantitatively, in which their abundance was found.

Introduction:-

Ayurveda is our Indian tradition: in which the virtues and religions of different types of medicinal plants have been explained. Our sages and sages were followers of scientific ideology, that is, they took Ayurveda to every house by making spirituality the basis for keeping all the plants with medicinal properties connected to the human society. Indian Vedanta, Nature and Medicine are worshiped like our mother. From the birth of man till his death, the contribution of Vedas, Nature and Vegeta is there, that is, through Vedas, Namkaran Sanskar and after death Sanskar, Vanaspati is used by man for life for the treatment of various diseases. Therefore, it becomes our supreme duty to protect the heritage of Ayurveda provided by our forefathers.

Experimental plant:-

Fig. *Salvia didinorum*

Scientific Classification:-

Kingdom: Plantae

Order: Lamiales

Spp. *S.didinorum*

Genus: *Salvia*

This medicinal plant is a hydrophyte. Various compounds have been isolated from this plant which are of great importance in drug designing. The growing time of this plant is March - April and oil seeds are grown. This plant is used in the treatment of various diseases and therefore natural properties have been seen in the leaves. The leaf stem of this plant has a large stem, apart from this, this plant also grows in the presence of less water. This plant bears flowers of different colors like red, white, purple, etc. The flowers of this plant are like an open mouth and their leaves are cut from the edge. Its length is 4-6 feet.

Part used:- Flower

Methodology:-

Primary Metabolites (Quantitative Estimation)

In the present study carbohydrate, proteins, phenols were extracted as primary metabolites.

Carbohydrates (Total soluble sugars) Extraction:

The dried experimental plant material (leaf & stem) in powdered form, 0.1g each was homogenized in pestle and mortar with 5ml of 80% ethanol and left overnight. Each sample was centrifuged at 12000 rpm for 15 min; the supernatant was collected separately and concentrated on a water bath using the method of (Loomis and Shull, 1973). Distilled water was added to make up volume to 50 ml and processed further for qualitative analysis.

Starch Extraction

The residual mass obtained after extraction of total soluble sugars of each of the test samples was suspended in 5 ml of 52% perchloric acid (Cready et al. 1950). Later, 6.5 ml of water was added to each sample and the mixture was shaken vigorously for 5 minutes.

Quantitative Estimation of Carbohydrate

1 ml of aliquot of each sample was used for the estimation of carbohydrates using the phenol sulphuric acid method of Dubois et al. (1951). A standard regression curve of standard sugar (glucose) was prepared. A stock solution of glucose (100 ug/ml) was prepared in distill water. From this solution, 0.1 to 0.8 ml was pipetted out into eight separate test tubes and volume was made up to 1ml with distilled water. These test tubes were kept on ice; 1 ml of 5% phenol was added in each tube and shaken gently. 5 ml of conc. sulphuric acid was added rapidly poured so that the steam hits the liquid and tubes were gently shaken during the addition of acid. Finally the mixture was allowed to stand on water bath at 26-30 °C for 20 minutes. The characteristics yellow orange color was developed. The optical density was measured at 490 nm using spectrophotometer.

Proteins Extraction

The test sample 0.1 g each (leaf & stem) were separately homogenized in 10 ml of cold 10% trichloroacetic acid (TCA) for 30 min; and kept at 4ºC for 24 hours. These mixtures were centrifuged separately at 15000 rpm for 10 min and supernatant were discarded. Each of the residues was again suspended in 10 ml of 5% TCA and heated at 80ºC on a water bath for 30 min. The sample was cooled, centrifuged and supernatant of each were discarded. The residue was then washed with distilled water, dissolved in 10 ml of 1N NaOH, and left overnight at room temperature.

Quantitative Estimation

10% TCA was used for protein extraction according to methodology of Osborne, (1962). 0.1 ml of sample was mixed with 3 ml 10% TCA, centrifuged at 15000 rpm for 10 minutes, to the pellet 10 ml 5% TCA was mixed and vortexed. Now it was taken in a test tube and incubated at 80ºC for 30 minutes, after incubation cooled it and took 1 ml sample from it and added 5 ml alkaline solution with 1 ml Folin & Ciocalteu's reagent and incubated again for 10 minutes at 37ºC or room temperature. Absorbance was read at 750 nm (wavelength)

against 10% TCA reagent blank. The analysis was performed in triplicates and the results were expressed mg/g dry weight sample.

Lipids Extraction

Distilled water was used for lipid extraction according to methodology of **(**Jayaram, 1981). Taken 0.3 g sample with 10 ml distilled water crushed it with the help of mortar pestle. Added 20 ml chloroform ($CHCl_3$) with 10 ml methanol (CH_3OH). The mixture was thoroughly mixed and left overnight at room temperature in dark for complete extraction. Later, 20 ml of chloroform mixed with 2 ml of water were added and centrifuged. Two layers were separated the lower layer of chloroform, which contained all the lipids, was carefully collected in the pre-weighed glass vials and the colored aqueous layer of methanol which contained all the water soluble substances and thick interface layer were discarded in each test sample. The chloroform layers dried and weighed.

Phenol Extraction

80% ethanol is used for the extraction of total phenol content in each sample was estimated by spectrophotometer method of (Bray and Thorpe, 1954). Took 0.2 g sample with 4 ml 80% ethanol, crushed it with the help of mortar and pestle. Centrifuged at 10000rpm for 10 min and collect supernatant and take 1 ml of sample added 1 ml of Folin & Ciocalteau reagent and incubated at room temperature for 3 minutes. After 3 minutes 2 ml of 20% sodium carbonate ($Na_2 CO_{3)}$ was added, mixed well and incubated the tubes in boiling water bath for 1 minute. Cooled rapidly and read absorbance at 750 nm (wavelength) against reagent blank. The analysis was performed in triplicates and the results were expressed as mg/g sample.

<u>Secondary Metabolites Extraction</u>

Flavonoids Extraction

Different plant parts (leaves and stem) were air dried and powdered separately. Each of these

Were extracted separately with 80% methanol in incubator (Subrmanian & Nagarajan, 1969) for 24 hours. The methanol soluble fractions were filtered, concentrated in vacuum and aqueous fractions were fractioned by sequential extraction with petroleum ether (Fr I), diethyl ether (FrII) and ethyl acetate (Fr III) separately. Each step was repeated thrice for complete extraction; fraction I was discarded in each case because it contained fatty substance, whereas fraction II and fraction III were concentrated and used for determining flavonoids. Fraction III was further hydrolyzed by refluxing with 7% sulphuric acid, filtered and filtrate was extracted thrice with ethyl acetate. All ethyl acetate layers were pooled separately; neutralized by distilled water with repeated washings and concentrated in vacuum. Both fraction II and III were taken up in small volume of ethanol (2-5 ml) before chromatographic examination.

Qualitative Estimation

Thin Layer Chromatography (TLC)

Thin glass plates (20x20 cm) were coated with Silica gel. The freshly prepared plates were air dried at room temperature; thereafter these were kept at 100ºC for 30 minutes to activate and then cooled at room temperature. The freshly prepared and activated plates were used for analysis.15 Each of the extract was co- chromatographed with authentic flavonoid as a marker for example quercetin, luteolin, kaempferol, and rutin etc. These plates were developed in an air tight chromatographic chamber saturated with solvent mixture (Benzene: Acetic Acid: Water:: 125:72:3) (Wong and Francis, 1968). The developed plates were air dried and visualized under UV light by exposure to ammonia fumes. The mouth of a 100 ml containing concentrated NH 4 OH was held in contact with each spot for about 5-10 seconds and fluorescent spots corresponding to that of standard markers were marked. The developed plates were also sprayed with 5% FeCl 3 , 0.1% alcoholic AlCl 3 and kept in I 2 chamber separately. The colored spots thus Developed were noted and the Rf value of each spot was calculated.

RESULTS AND DISCUSSION

The following were the results of types and amount of primary and secondary metabolites obtained from flower of ***Salvia didinorum***

Table 4.1: Primary metabolites extracts from ***Salvia didinorum*** flower (mg/gram dry weight).

Primary Metabolites	flower
	OD (A°)
Starch	0.211
TSS	0.223
Protein	0.372
Phenol	0.507
Lipid	0.550

The above table shows that the concentration of primary metabolite in flower. At a particular optical density the following results are calculated by the formula: Optical density x Calculation factor.

In flower of ***Salvia didinorum*** starch is 1.65mg/g, TSS is 0.7mg/g, Protein is 26.8mg/g, Phenol is 2.45mg/g, and Lipid is 6.0mg/g.

These results clearly show the presence of higher amount of proteins which is 26.8mg/gram in flower of ***Salvia didinorum.*** Other primary metabolites are present in minor quantity such as TSS which is very low in flower. Lipid content is found more in flower.

Starch and phenols content is very low in flower.

Based on the result it can be concluded that, the flower ethanolic extract of ***Salvia didinorum*** which contains high amount of primary metabolites as primary metabolites have no role in drug formulation but in future this plant can be used as a possible food supplement or in pharmaceutical industry.

Flavonoids:

Three spot of flavonoids were observed in flower of ***Salvia didinorum*** on thin layer chromatography plates developed and placed iodine chamber. The RF values of these spots matched with their respective authentic standards and were identified as Kaempferol, Luteolin, Quercetin. The Rf value was calculated by using the formula Rf = DS/DF.

DS = Distance travelled by Solute

DF = Distance travelled by Fragment

Table 4.7: Activity of flavanoids obtained from ***Salvia didinorum*** flower

Isolated compounds	Rf value			Physical appearance			Colour after spray		
	S1	S2	S3	Day light	UV Ammonia	I_2 vapour	R1		R2
							Visible	UV	Visible
Kaempferol	0.83	0.81	0.55	GN-YW	BT-YW	YW-BN	BN	BK	YW
Luteolin	0.78	0.81	0.77	GN-YW	YW	YW-BN	TN	BK	DL – YW
Querecetin	0.56	0.64	0.41	GN-YW	YW	YW-BN	BT-GY	BK	DL – YW

S1- Benzene: acetic acid: water (125: 72: 3), S2- n- Butanol: acetic acid: water (4: 1: 5), S3- Conc. Hydrochloric acid: acetic acid: water (3: 30: 10), R1 – 5% $FeCl_3$ solution, R2 – 5% alc. $AlCl_3$ solution, YW – Yellow, BK - Black, BN - Brown, BT - Bright, DL - Dull, GN – Green, GY – Gray.

In our present investigation we figure out the three spot of flavonoids were observed in flower of ***Salvia didinorum*** on thin layer chromatography plates developed and placed iodine chamber. The RF values of these spots matched with their respective authentic standards and were identified as Kaempferol, Luteolin, Quercetin. Solvent system Benzene: Acetic Acid: Water (125:72:3) gave best results with RF values viz, Kaempferol 0.83, Luteolin 0.78, Quercetin 0.56. When other solvents viz. n- butanol: Aceti Acid: water (4:1:5) and conc. HCL: Acetic Acid: Water (3:30:10), the RF value of Quercetin was found to be 0.64 and 0.41, Kaempferol was found to be 0.81 and 0.55 while that of Luteolin was found to be 0.78 and 0.77, respectively.

Conclusion

The medicinal values of a plant life in some chemical substances that produce a definite physiological action on the human body. Phytochemicals analysis is a paramount importance in identifying a new source of the therapeutically and industrially valuable compounds having medicinal plants have been chemically investigated. In the present investigation primary metabolites was quantitatively analysed using *Salvia* Flower. In the present study, quantitative analysis of flower ethanolic extract of *salvia* was investigated. The extract was found to possess more secondary metabolites and it exhibit radical scavenging activities. Based on the results it can be concluded that, the flower ethanolic extract of *salvia* which contains high amount of secondary metabolites and exhibits free radical scavenging activities.

Since the use of flavonoids are the potential candidates of bioactive agents in pharmaceutical and medical sectors to promote human health, prevent and cure various diseases. So we can conclude that, this plant may offer a great scope for the drug development in future.

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