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Research Article

Characterization of Flavonoids in Aqueous extract of Desmodium gangeticum by RP-HPLC

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Abstract: Flavonoids and the other phenolic compounds are commonly known as plant secondary metabolites that hold an aromatic ring bearing at least one hydroxyl groups. Flavonoids are principal active constituents have been used to treatment of various human diseases. The plant *Desmodium gangeticum* (DC), Family-Fabaceae has been used in folklore medicine in the treatment of various ailments. Many of the Ayurvedic formulations contain this medicinal plant and considered as a Master of Medicinal Plant in Ayurveda due to its wide uses in formulations. A medicinal benefit includes bitter tonic, febrifuge, digestive, anti-emetic, antipyretic & anti-inflammatory activity. The chromatographic separation was achieved by using a C-18 column with dimension of 4.6 mm I.D.X 250 mm and particle size of 5µm. The mobile phase contain methanol: water (70:30). The flow rate was 0 .5 mL/min, and a column temperature of 25°C. The injection volume was 25µl, and UV detection was achieved at 254 nm. Effective separation and quantification was achieved in less than 10 min. The method was simple, accurate, precise and could be successfully applied for the characterization of flavonoids in aqueous extract of DC.

Keywords: *Desmodium gangeticum* (L.) DC, Flavonoids & RP-HPLC.

INTRODUCTION

The World Health Organization has also recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines. Proven agro-industrial technologies need to be applied to the cultivation and processing of medicinal plants and the manufacture of herbal medicines (Himesh, S. *et al.,* 2011) . Flavonoids consist of a huge group of polyphenolic compounds having a benzo-γ-pyrone structure and are universally present in plants. They are synthesized by phenylpropanoid pathway. As a dietary component, flavonoids are thought to have health-promoting properties due to their high antioxidant capability. They have ability to induce human protective enzyme systems. The number of studies has recommended protective effects of flavonoids against many infectious (bacterial and viral diseases) and degenerative diseases such as cardiovascular diseases, cancers, and other age-related diseases (Kumar, S., & Pandey, A. K. 2013).

Desmodium gangeticum (DC) commonly known as salpan, salvan and sarivan in Hindi; belonging to family-Fabaceae. Salparni is found throughout tropical India into the lower portions of the Himalayans range, and it related species are also found in regions of China (*Desmodium styracifolium, Desmodium pulchellum)*. The meaning of its Sanskrit name 'Leaves like sala' suggests that its leaf structure is similar to those of the tree Shorea robusta. Its synonyms are Aakuparnijaa, Amshumati, Atiguha, Atiruha, Deergmoolika, Dhurva, Guha, Mahaakleetaanika, Parninee, Peethanee, saumya, Sthira, Triparni, vidyarigandha (Kirtikar, K. R., & Basu, B. D. 1996).

Desmodium gangeticum

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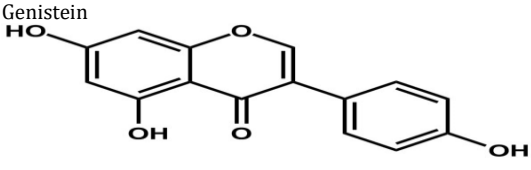
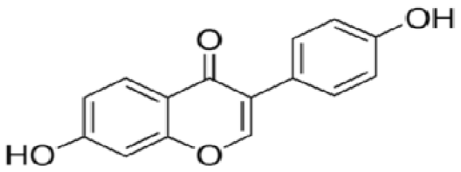
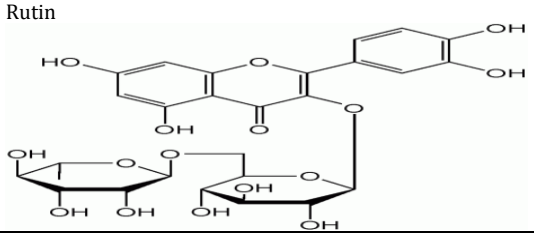
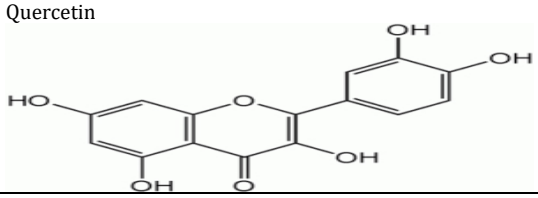
Major flavonoids characterized by RP-HPLC in the aqueous extracts DC

S.No.	Flavonoid	Description
1.	Genistein  Fig. 1. Chemical structure of genistein	Genistein [4′,5,7-trihydroxyisoflavone or 5,7-dihydroxy-3-(4-hydroxyphenyl) chromen-4-one] ($C_{15}H_{10}O_5$) belongs to a multifunctional natural isoflavonoid class of flavonoids with a 15-carbon skeleton. The chemical structure of genistein is similar to estradiol (Spagnuolo, C. *et al.,* 2015). Genistein is a common form of phytoestrogens that are found in a variety of plants, especially in soy. Phytoestrogens are a group of plant substances that have a chemical structure similar to estrogen, exerting estrogenic and antiestrogenic effects (Ganai, A. A., & Farooqi, H. 2015).
2.	Daidzein 	Daidzein 7-O-beta-D-glucoside is a glycosyloxyisoflavone that is daidzein attached to a beta-D-glucopyranosyl residue at position 7 via a glycosidic linkage. It is also called phytoestrogen due to its structural similarity to the human hormone estrogen. Daidzein is reported to play a significant role in the prevention and treatment of a variety of diseases such as cancer, cardiovascular disease, diabetes, osteoporosis, skin disease, and neurodegenerative disease (Meng-Yao Sun *et al.,* 2016).
3.	Rutin 	Rutin is a flavonoid present in the plant kingdom as Allopathic substances. Rutin is the rhamnoglucoside of the flavonoid quercetin and found in many plants and used for treatment of various diseases related to the vascular. It is quercetin-3-rutinoside or 3, 3',4', 5,7-pentahydroxy flavones-3-rutinoside, and has a chemical formula $C_{27}H_{30}O_{16}$ (Soni, H. *et al.,* 2013).
4.	Quercetin 	Quercetin is 3,3',4',5,7 -pentahydroxyflavanone (or its synonym3,3',4',5,7-pentahydroxy-2-phenylchromen-4-one). It is widely used in medicine and pharmaceutics. In particular, it is used for cancer treatment; as it restrains the growth of cancer cells (Kumar, R. *et al.,* 2017).

In the present investigation an attempt was made to characterize various flavonoids in aqueous extract DC by RP-HPLC method. The method was simple, accurate, precise and could be successfully applied for the analysis.

MATERIALS AND METHOD

Collection and Authentication

Aerial parts of *Desmodium gangeticum* were collected from herbal garden of Dehradun (Green Biotech). The plant was identified and authenticated at the Botanical Survey of India (BSI), Northern regional centre, Dehradun with the accession number BSD-112743.

Preparation of Plant Extracts

The powder was subjected to successive soxhlet extraction with different solvents in increasing order of polarity at different temperature (i.e. Petroleum Ether <Benzene< Chloroform< Acetone< Ethanol<Chloroform water I.P.

Extraction Procedure

About 200 gm of accurately weighed dried powder was taken in thimble. About 2.5 lit .of solvent taken in a round bottle flask and fitted with thimble and condenser on a heating mental and extracted for 24 hours. On completion of extraction the drug was taken out from the thimble and dried in shed. Then the residue was extracted with other solvents successively in the same manner. The extracted drug was taken in a china dish and the solvent was evaporated on steam bath and finally reduced to dryness to get dry extract and transferred to previously weigh airtight glass container, weighed on an electronic balance and stored in refrigerator. Further due enormous literature survey flavonoids were characterized from aq.extract DC by RP-HPLC method.

Determination of Total Flavonoids Content

The content of total flavonoids was determined by aluminum chloride colorimetric method as quercetin equivalent. Plant extract (10 mg/ml) in respective solvent (stock solution) was mixed with 2 ml $AlCl_3$ (2% w/v) in methanol and the solution was made up to 25ml with methanolic solution of acetic acid (0.5% v/v) (Probe solution PS). 1ml of SS was madeup to 25ml with methanolic solution of acetic acid (contrast solution CS).The absorbance of PS and SS was measured at 420nm after 30 min. The results were expressed as % of total Flavonoids content (Himesh, S. O. N. I. *et al.,* 2012).

%TFC = Absorbance at 420 x dilution x 100 / $E^{1\%}_{1\,cm}$ x wt. of extract in gm

HPLC Analysis

RP-HPLC analysis was carried out using a LC-100, Cyberlab TM , Salo Torrace, Millburry, MAO 1527, USA with LC-UV-100 UV detector. A CAPCELL (C-18) HPLC-packed column (4.6 mm I.D.X 250 mm), type MG 5 µm, number AKAD/05245 was used for the chromatographic separations. The mobile phase contain methanol: water (70:30). The flow rate was 0.5 mL/min, and a column temperature of 25°C. The injection volume was 25µl, and UV detection was achieved at 254 nm.

RESULT AND DISCUSSION

The total flavonoid content of aqueous extract of DC was determined by colorimetric method and it was found to be 2.01(%TFC).The best result of RP-HPLC method for the simultaneous determination of flavonoids from aqueous extract of DC were obtained by using a C-18 column with dimension of 4.6 mm I.D.X 250 mm and particle size of 5µm. A mixture of methanol: H_2O (70:30). The flow rate of 0.5mL/min. The effluent was monitored at 254 nm. Under the described experimental

conditions, the flavonoid of aqueous extract of DC was analyzed. The result was tabulated in table 2 & Fig.1. Te retention time (RT) of various flavonoid was compared with standard Fig.1-5). The RT of major bioactive Rutin, Quercetin, Genistein and Daidzein was found to be 5.482, 7.96, 9.47 & 11.001 respectively. The construction of chromatographic fingerprints plays an important role in the quality control of complex herbal medicines. Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines (Himesh, S. *et al.,* 2011). Thus chromatographic fingerprint should be considered to evaluate the quality of herbal medicines globally considering multiple constituents present in the herbal medicines (Soni, H. *et al.,* 2012).

CONCLUSION

The study also conclude that the plant have rich sources of phytonutrients compounds. Flavonoids have numerous biochemical and antioxidant effects. A simple, reproducible and efficient method for the determination of flavonoid of DC was developed. The method was simple, accurate and precise and could be successfully applied for the analysis.

Table 1: Total Flavonoid Content

S.No	Sample	%TFC
1.	Aqeous extract of *DC*	2.01

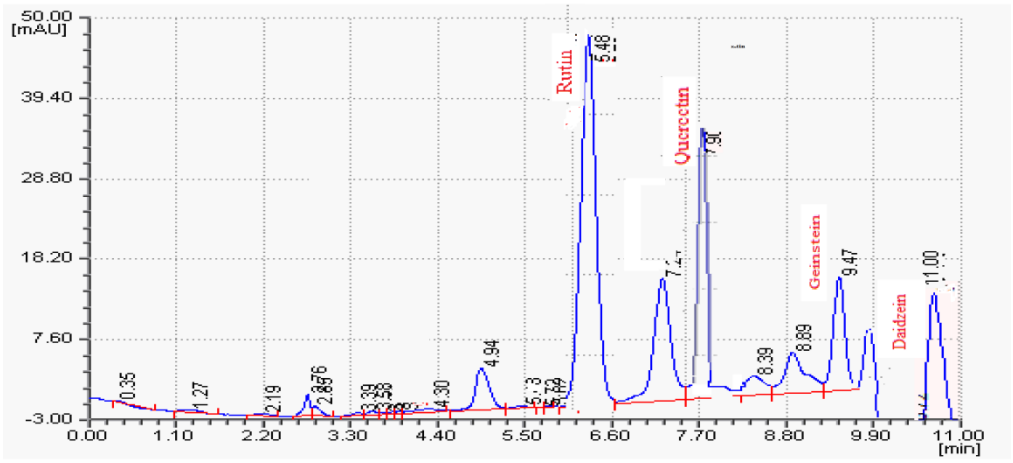


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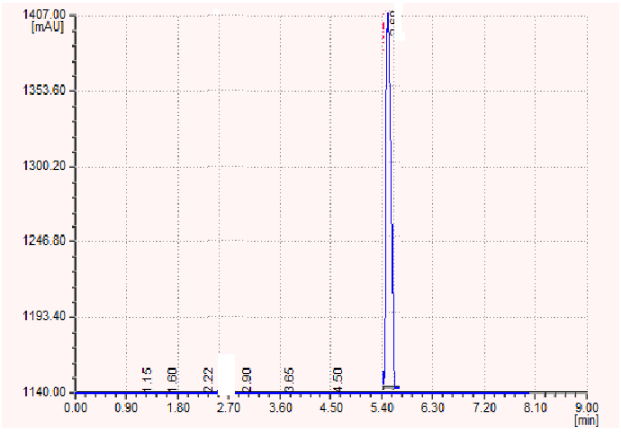
Fig.1 HPLC analysis of Aqueous extract of DS



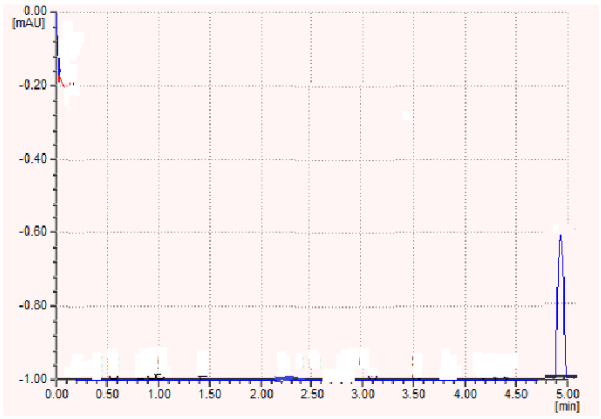
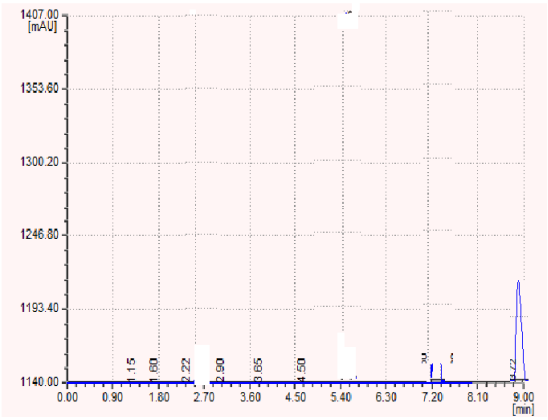


Fig.2 HPLC chromatogram Standard (Quercetin) Fig.3 HPLC chromatogram Standard (Rutin)



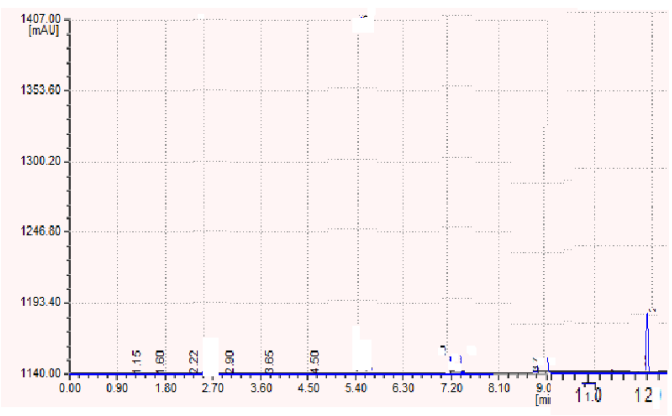


Fig.4 HPLC chromatogram Standard (Genistein) Fig.5 HPLC chromatogram Standard (Daidzein)

Table 2: HPLC ANALYSIS of Aqueous extract of DS

S.No	Vitamin	RT(min)	Height	Area	Conc.	Half width	Res	Theo.Plate	Tail.Factor
1		2.7602	141150	2623167.1	40.012	18.58	0.77	390.73	1.53
2		4.949	233	2616.8	69.6570	11.23	2.21	1282.43	1.61
3	Rutin	5.482	2828	97982.7	56.7556	48.79	0.98	71.68	1.16
4		7.770	5572	70158.6	1.9125	12.22	0.71	1569.90	1.28
5	Quercetin	7.96	923	6293.2	3.6687	11.15	3.69	1297.44	1.60
6	Genistein	9.472	12150	89567.0	64.0015	14.78	1.30	1761.55	3.29
7.	Daidzein	11.001	3572	70158.6	1.7125	102.22	0.76	1569.90	1.68

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