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Quality assessment and phytochemical profile of *Capparis decidua* (Forssk) Edgew

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Abstract
Capparis decidua a xerophytic shrub of family Capparidaceae is of great nutritional and medicinal value. Its fruits (capers) are used as a vegetable and to cure various ailments. In the present work, fruit sample was studied macroscopically, microscopically and analyzed for phytochemicals present in it using analytical techniques like high performance liquid chromatography (HPLC). Various parameters were studied as per the World Health Organization recommended methods of quality control. The heavy metal analysis was done by Atomic Absorption Spectrophotometer (AAS) and pesticide residue was determined by GC-MS. Transverse section of fruit showed the presence of exocarp, mesocarp with vascular bundles, endocarp, endosperm, and embryo. Powder microscopy of fruit illustrated parenchymatous mesocarp cells, fibers, spiral vessels, and mucilage. Alkaloids, carbohydrates, proteins, sterols and phenolic compounds including flavonoids were qualitatively assessed. Trace amount of heavy metals were identified by AAS. Six polyphenols were determined by HPLC. GC-MS analysis identified traces of non-toxic pesticides in the ethyl acetate extract. Outcomes of the above study can be used for making diagnostic indices for the identification and authentication of the herb and can also be helpful for further exploring the pharmacological potency of the herb.

Keywords: *Capparis decidua*, GC-MS, Hypolipidemic activity, HPLC, microscopy, morphology

1. Introduction
According to the World Health Organization (WHO), approximately 80% of the population of developing countries uses traditional medicine for their healthy livelihood. Medicinal plant constitutes the backbone of the traditional system of medicine. The medicinal plant includes the fresh or dried plant, part, whole, chopped or powdered form and plant extract in various solvents. WHO has concluded that around 20,000 medicinal plants are being used in the pharmaceutical industry or in folk medicine [1]. Now a day's herbal medicinal system is being preferred over the conventional medicinal system as they are inexpensive, easily available and has least/no adverse effect during the treatment. In this respect a herb commonly famous as teent or kair and scientifically known as *Capparis decidua* a shrub of the family Capparidaceae is being studied as it has high nutritional value and medicinal effects [2, 3]. The abundance of various physiologically active constituents makes *C. decidua* beneficial for mankind. Traditionally bark of the plant has been used in boils, cough, asthma, inflammation and root is helpful in fever management, as anthelmintic and purgative, leaves are used in pyorrhoea. Wood coal is useful in muscular injuries. The plant is also used in rheumatism, liver infections, ulcer, piles, renal disorders, diarrhoea, febrifuge, skin diseases, constipation, lumbago, toothache, dysentery and cardiac troubles. The plant also acts as aphrodisiac, analgesic, alexipharmic, emmenagogue tonic, purgative, and appetite enhancer. In Sudan, *C. decidua* is useful in swelling, jaundice and infection of joints. In Unani system, leaves are beneficial as an appetizer, in cardiac troubles and in biliousness. In combination with shoots of *Peganum hermala* shoots of *C. decidua* are used as anti-fertility drug [4]. The shrub is used not only in traditional medicine but also as food, its flowers are taken in vegetable form and the fruits are eaten as pickle, due to their nutritional properties and bioactivity. Various pharmacological activities like insecticidal, anti-aging, anti-arthritis, anti-microbial, antiviral, anti-atherosclerotic, anti-inflammatory, analgesic, and nociceptive activities are also attributed to various parts of *C. decidua* [5]. Presence of carbohydrates, protein, fibre and minerals including calcium, potassium, zinc, manganese, iron makes this plant a nutritional food. It is rich in vitamin C and is the richest source of beta carotene, moreover the oil content of the seed, sugar and protein content of flower contributes to its nutritional values [6].

In the current era ethnobotany study of flora is gaining much attention as the compounds of natural origin are comparatively safer than the synthetic ones. Such medicinal and nutritional important plant deserves the standardisation for their quality control and monograph of such medicinally active herb needs to be developed. Moreover, standardization of herb is also necessary for its error-free authentication. In the present paper, various standardisation parameters and safety profile of *C. decidua* fruit have been discussed.

2. Materials and Methods

2.1 Materials

2.1.1 Collection and authentication of plant material

Fruits of *C. decidua* were procured from local market Hisar. Authentication was done by Dr. Anjula Pandey, Principal Scientist, ICAR- National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, India (letter no. NHCP/NBPGR/2017-7). A voucher specimen has been submitted in the Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana for future reference.

2.1.2 Chemicals and instruments

Analytical grade hydrochloric acid, phloroglucinol, glycerine, hematoxylin, eosin, ethanol, ethyl acetate, petroleum ether (60-80ºC), nitric acid, perchloric acid, sulphuric acid, reagents for phytochemical analysis (Dragondorff's reagent, Wagner's reagent, Hager's reagent, Mayer's reagent, Millon's reagent, Molisch's reagent, Benedict's reagent, Ninhydrin reagent, Fehling's reagent, ferric chloride, lead acetate, silver nitrate, ammonia, magnesium metal, vanillin, ninhydrin, acetic anhydride, acetic acid, chloroform), biological kits from Erba Diagnostics were used in the study. HPLC grade acetonitrile, formic acid, ethanol and methanol were purchased from HPLC Lab reagents Mumbai. All the chemicals were purchased from Hi-media, Mumbai, India. Atomic absorption spectrophotometer (GBC 932 plus, Shimadzu Europe), GC-MS-QP2010 Plus (Shimadzu, Kyoto, Japan), Motic compound microscope BA200, Muffle furnace, Hot air oven, Soxhlet apparatus. HPLC (Water's Acquity Ultra HPLC) were used.

2.2 Methods

2.2.1 Pharmacognostic characterization

2.2.1.1 Morphological and Microscopical studies

The morphological/macroscopic study of the plant part was performed by naked eye/magnifying lens. Organoleptic characters such as shape, size, surface, colour, odor and taste etc. were recorded [7]. For microscopic studies, permanent slides of the transverse sections of fruit were prepared after fixing the sample in wax and then 5-6µ thick sections were cut using microtome. For powder microscopy dried plant material was ground in a grinder and passed through sieve no. 40. A pinch of powder was taken on the slide. The transverse section and powder was stained with phloroglucinol, concentrated hydrochloric acid and mounted with glycerine. Microscopic examination of the fruit sections and powdered fruit was done using a compound microscope. The photomicrographs of all necessary cells and tissues were taken at different magnifications [8, 9].

2.2.1.2 Physicochemical Evaluation

WHO guideline was followed to study various physicochemical parameters of the fruit [10]. Parameters like foreign matter, moisture content, crude fiber content, ash value, extractable matter, swelling index were studied.

2.2.2 Preparation of plant extract

The fruits were dried under shade, coarsely powdered using pestle and mortar. The crushed fruits were defatted with petroleum ether (60-80ºC) and extracted by continuous hot percolation using soxhlet assembly at room temperature for 72 h using ethyl acetate as solvent. After filtration, the extract namely ethyl acetate extract (EAE) was concentrated at 45°C under reduced pressure in a rotary evaporator to obtain crude semisolid mass which was kept in a desiccator for further use.

2.2.3 Safety profile

2.2.3.1 Heavy metal analysis

The heavy metal content was determined in ppm/g of the crude powdered drug using AAS. An AAS with hollow cathode lamp for various heavy metals was used. The standard calibration curves were prepared and the instrument was optimized as per requirement. For the sample preparation dried crude plant material (1g) was kept in nitric acid (10 ml) for 12 h, heated with 3 ml of perchloric acid until nitric acid gets evaporated. After cooling the above solution dilution was done with 20% hydrochloric acid to 50 ml and analyzed after calibrating the instrument. Calibration curve of different dilutions of different metals was prepared and taken as the reference to investigate the unknown sample [10].

2.2.3.2 Pesticide residue analysis by GC-MS

The sample was prepared by dissolving the extract in the respective solvent (ethyl acetate) and filtered through 0.4 µ syringe filter. Data was collected in the form of peaks; these peaks were matched with commercial libraries of WILEY8.LIB, NIST08.LIB, NIST08s.LIB, NIST11.lib, and PESTEI_3.lib collections of pesticides. Pesticide residue of the extract was obtained as percentage area of the total peaks present.

2.2.4 Phytochemical profile

2.2.4.1 Preliminary phytochemical screening

The drug extract was tested for the presence of various phytoconstituents viz. alkaloids, glycosides, carbohydrates, phytosterols, proteins, phenolic compounds including tannins and flavonoids [11].

2.2.4.2 HPLC Analysis of the extract

As per the literature *C. decidua* has a very good quantity of polyphenols in its various parts. In the present work using HPLC (high-performance liquid chromatography) various polyphenolic compounds were determined in the ethyl acetate extract of the plant fruit.

Standard solutions: Stock solution of rutin (50 ppm), quercetin (50 ppm), apigenin (50 ppm), gallic acid (50 ppm) and catechin (50 ppm) were prepared in HPLC grade methanol, filtered using 0.45 µm cellulose syringe filter. The standard solutions were stored at 4 ºC prior to use.

Preparation of Sample Solution: 500 ppm stock solution of the extract was prepared in HPLC grade methanol. The sample solution was then filtered from 0.45 µm cellulose syringe filter.

Ultra HPLC Instrumentation and Conditions: Water's Acquity Ultra HPLC (Ultra high-pressure liquid

chromatography) with attached quaternary pump and Acquity PDA detector. The column used was water's BEH C18 having 2.1 mm diameter, 50 mm length and 1.7 µ particle size. The temperature of column was set at 22 ºC. The mobile phase used was formic acid (A) and acetonitrile (B) at gradient flow at a flow rate of 0.5 ml/min. Programming of gradient ramping is shown in Table 1. Injection volume was 1 µl. Separation of solutes was performed at 275 nm for a period of 9 min. Identification of peak was based on retention time (Rt) against the chromatogram of standards. Data was analyzed by Water s Empower software.

Table 1: Programming-gradient ramping of HPLC

Time (min.)	Flow rate (ml/min)	% A	% B	Curve initial
initial	0.5	95	5	Initial
6	0.5	70	30	7
6.5	0.5	70	30	7
7	0.5	10	90	6
8	0.5	10	90	6
8.1	0.5	95	5	6
10	0.5	95	5	6

3. Results & Discussion

3.1 Morphology and Microscopic characters of fruit

Standardisation studies are essential for the raw as well as the finished product for the authentication as the authentication herbals may show poisonous or allergic effects. [12]. The morphological examination is the simplest and quickest means to establish the identity and purity of a particular drug [7]. In the present study it was observed that the fresh fruits were ovoid, the outer texture was globulous, green to red in colour and cherry shaped (Fig. 1). Size of fruit was 1-2 ± 0.034 cm in diameter. The fruit was having 8-10 seeds embedded in the mesocarp. The white seeds were spherical in shape. Fruit had an astringent odour with bitter and mucilaginous taste. Microscopic study is the anatomical study of the plant part as whole or powder. On the basis of microscopy, many characteristic features of a plant part such as various layers of the cell and their types, oil glands, trichomes, vascular bundles, seed, pollen grain morphology can be analyzed which could help to achieve the goal of standardization of the herb. The basis of microscopy for standardization has been published by. The pharmacognostic standardization i.e., quality control methods [13] consists of various microscopic [7, 14]. The transverse section of mature fruit exhibited exocarp, mesocarp with vascular bundles, endocarp, endosperm and embryos. The mesocarp is constituted by parenchymatic cells and vascular bundles. The endocarp (testa) is parenchymatous with thickened walls and by elongated sclerenchymatous fibres. The embryo is ellipsoid and slightly curved, it is constituted of two fused cotyledons (Fig. 2). The microscopy of powder showed the presence of mucilaginous mesophyll parenchymatous cells, fibre, spiral xylem vessels and mucilage (Fig. 3).

Fig 1: *Capparis decidua* fruit

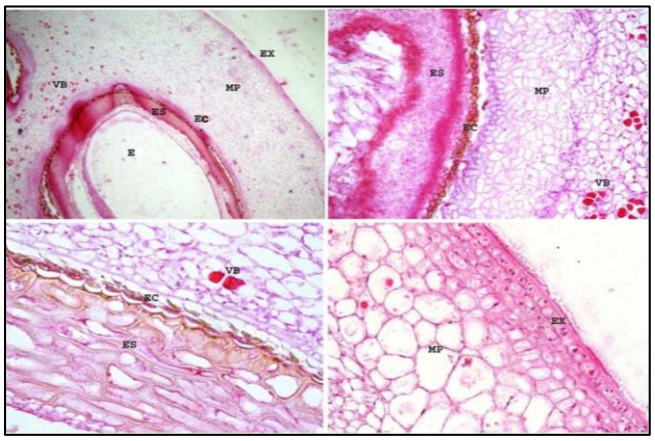

Fig 2: Transverse section of fruit of *C. decidua*, Hematoxylin –Eosin stained ×40, **E:** Embryo, **EC:** Endocarp, **ES:** Endospermic cells, **EX:** Exocarp, **MP:** Mesophyll, **VB:** Vascular bundle

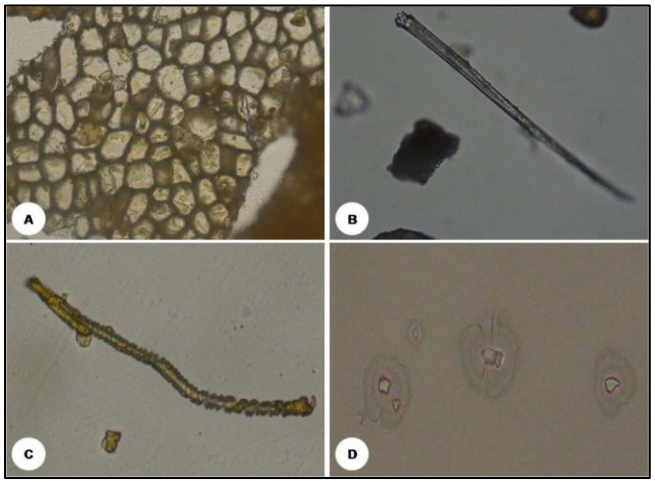

Fig 3: Powder microscopy of fruit of *C. decidua* (Forssk.) Edgew, phloroglucinol-HCL stained X40 **A:** Parenchyma cells, **B:** Fibre, **C:** Xylem vessel, **D:** Mucillage

3.2 Physicochemical parameters

Physicochemical parameters are useful for determining the purity of the drug. These parameters have constant values for a herb and any change in these values is the indication of the spurious or adulterated drug. Foreign matter is the part or organ other than the plant part with therapeutic value or any organism, any mineral admixture like soil, stones, dust or sand etc. Herbal materials must be entirely free from such foreign materials. The plant material procured from the market is seldom free from these materials therefore limit has been set for each herb in its monograph. Presence of foreign matter can be easily determined through macroscopic examination. The foreign matter content in the sample was 0.41± 0.071%. In a crude drug, the percentage of active chemical constituents is mentioned on the air dried basis. Hence in order to control the moisture content of a drug it should be determined. Moreover, an excess of moisture may lead to chemical change and encourages microbial growth, fungi, insects, leading to the decomposition of plant drugs. The loss on drying was found to be 6.12 ± 1.133% of the drug. Several health benefits like maintenance of healthy laxation, the reduced risk of cardiovascular disease and cancer etc. are related with adequate dietary fiber intake. The fruits of *C. decidua* are used for nutritional and medicinal purpose. Therefore the crude fiber content was determined and found to be 2.02 ± 0.562%. Quantitative estimation of ash value

estimates the quality and purity of the crude drug. It includes total cash value, acid-insoluble ash and water-soluble ash Total ash content is an indication of the total inorganic compound derived from plant tissue. Foreign material is determined as acid insoluble ash as it depicts the silica/earthy material present in the drug. Water soluble ash determines the quantity of inorganic components. Total ash results due to loss of organic material in form of carbon dioxide and leaves behind inorganic components. The total ash was found out to be 6.15 ± 0.611%. Acid-insoluble ash and water soluble ash value were determined as 2.5 ± 0.701% and 9.12 ± 0.141% respectively. Extractive values determine the amount of the active constituents in plant material and their solubility in a particular solvent. It is also an indication whether the crude drug is exhausted or fresh [7]. This method is used for materials which do not have any suitable chemical or biological assay for determining the quantity of active constituents. Alcohol soluble and water-soluble extractive value was found out to be 31.14 ± 2.512 and 19.7±1.063 mg/g respectively. Swelling property of many herbs is the reason for their therapeutic potential. Gums, mucilage, pectin or hemicelluloses present in medicinal plant shows a number of pharmaceutical utility and they have the characteristic feature of getting swelled in water. Basically, swelling index is the volume in ml taken up by the swelling of 1.0 g of plant material under specified conditions. In our investigation, the swelling index was found to be 2.8 ± 0.212ml/g. [15]. (Table 2).

3.3 Safety profile

Herbal medicines have a prominent role to play in the pharmaceutical and health market and are used as the first choice in self-treatment for minor illness; making the safety of herbal products an important public health issue [16]. WHO recommends that medicinal plants should be checked for the presence of heavy metals and pesticides residue? Usually soil is subjected to contamination either through atmospheric like industrial activities, wastewater, rainfall, atmospheric dust and agricultural practice, like spraying during farming and throughout storage [11, 17].

3.3.1 Heavy metal analysis

Heavy metal content in plant material is dependent on extrinsic factors like the soil and treatments etc. They are important for the safety profile and risk analysis of the drug thus should be studied as safety profile parameter. High levels of heavy metals in the herb are hazardous as they are non-biodegradable. These toxins accumulate in different organs and may disturb the normal functions of central nervous system, liver, lungs, heart, kidney, brain and produce serious health hazards such as injury to the kidneys, symptoms of chronic toxicity, renal failure, and liver damage [18] and sometimes different types of cancers, skin eruptions, intestinal ulcers, etc [19]. WHO regulates the maximum permissible limits of toxic metals [20, 21]. The heavy metals contaminate the drug in one or the other way similar to environmental pollutants or pesticides. The studied drug contained chromium, nickel, iron, zinc, cadmium, copper, manganese, lead and cobalt but their concentrations were within the permissible limits as recommended by WHO (Table 2), thus it can be concluded that the fruit extracts are suitable for human consumption.

Table 2: Heavy metal content in fruit powder of *C. decidua*

Element	Concentration detected (ppm)
Chromium	0.3137
Mercury	0.0414
Arsenic	0.3015
Nickel	0.0776
Iron	3.4263
Zinc	3.0588
Cadmium	0.0269
Copper	0.3287
Manganese	0.4902
Lead	0.4894
Cobalt	0.0116

3.3.2 Pesticide residue analysis by GC-MS

Use of pesticides during cultivation makes the herb harmful for use as these pesticides are just like poison to the human body. The residues of pesticides, their metabolites and/ or degradation products will remain in plants, or in the soil that becomes a notable source of contamination for herbal. In the present investigation, the pesticide residue was analyzed using GC-MS which is a coupled technique of chromatography and spectroscopy. Pesticide residue found in the extract is shown in Table 3. Major pesticides found in EAE are jasmolin II, triademinol, demeton-S, cinerin-I, oxamyl, di-allate-1. Pesticide residue analysis revealed that the extract had a trace quantity of pesticides reported as harmless.

Table 3: Pesticide residue in the ethyl acetate extract of *C. decidua* fruit

Ethyl acetate extract			
Peak	**Retention Time**	**Area% Name**	**Name**
1	4.621	0.17	Aldicarb
2	4.803	0.74	Thiocyclam
3	4.923	0.75	Molinate
4	5.016	0.45	Hymexazol
5	5.531	0.04	Propoxur
6	5.885	0.18	Oxabetrinil
7	7.137	0.07	Esprocarb
8	8.457	2.36	Di-allate-1
9	8.713	0.28	Methidathion
10	9.169	1.79	Oxamyl
11	9.957	0.29	Tri-allate
12	10.562	0.40	Bromobutude-debromo
13	12.167	0.23	Promecarb
14	12.762	0.18	Isoprocarb
15	14.841	0.33	Molinate
16	15.533	0.48	Methoprene
17	16.318	0.25	Methiocarb sulfoxide
18	16.573	0.13	1,2-Benzenedicarboxylic acid

19	16.695	0.25	Xylylcarb
20	17.521	0.32	Prohydrojasmon-1
21	18.395	0.12	Prohydrojasmon-2
22	19.494	0.11	Mefenacet
23	20.576	0.27	Jasmolin I
24	21.784	13.76	Triadimenol
25	22.003	3.58	Demeton-S
26	23.967	2.67	Cinerin I
27	24.540	0.48	Cyromazine
28	25.956	0.19	Cycloate
29	28.128	0.42	Cinmethylin
30	30.927	0.37	Allethrin-2
31	34.638	1.81	Jasmolin II
32	34.931	0.61	Pyrethrin I

3.4 Phytochemical profile

Qualitative phytochemical analysis revealed the rich chemical potential of the drug. Fruit extract has shown the presence of alkaloids, carbohydrates, proteins, sterols, phenolic compounds; tannins and flavonoids. Qualitative phytochemical analysis supports the multiple traditional and folk uses of *C. decidua.*

3.4.1 HPLC analysis of extract

HPLC method was successfully developed to identify polyphenols. The developed method of simultaneous determination of the polyphenols by HPLC is simple, cost effective and reproducible with very less run time. Result has shown good separation of six polyphenols in ethyl acetate extract of *C. decidua* fruit shown in chromatogram (Fig. 4). For identification Rt (retention time) of different peaks were compared with that of reference compound Rt. Six polyphenols rutin, catechin, quercetin, genistin, gallic acid and ellagic acid were identified in the extract (Table 4). Overlay spectra of the extract and the standard polyphenols is given (Fig. 5 A-F).

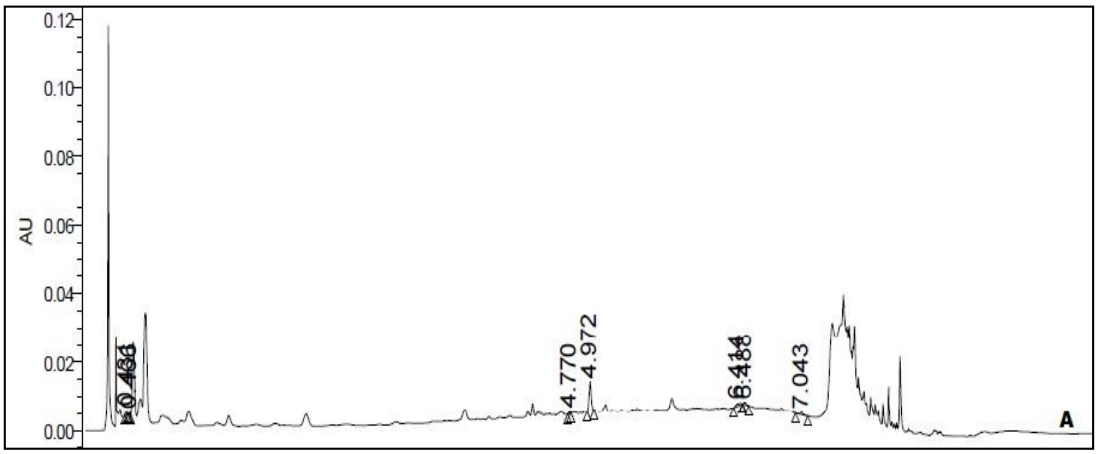

Fig 4: HPLC chromatogram of extract

Table 4: Rt and% Area of detected polyphenols

S. no	Reference Standard	Rt (min)	Peak no.	% Area
1	Rutin	4.985		
	Plant Extract	4.972	3	66.50
2	Catechin	6.42		
	Plant Extract	6.414	4	11.84
3	Quercetin	6.500		
	Plant Extract	6.488	5	6.32
4	Genistin	6.975		
	Plant Extract	7.043	6	5.81
5	Gallic acid	0.432		
	Plant Extract	0.431	1	5.29
6	Ellagic acid	4.729		
	Plant Extract	4.770	2	2.43

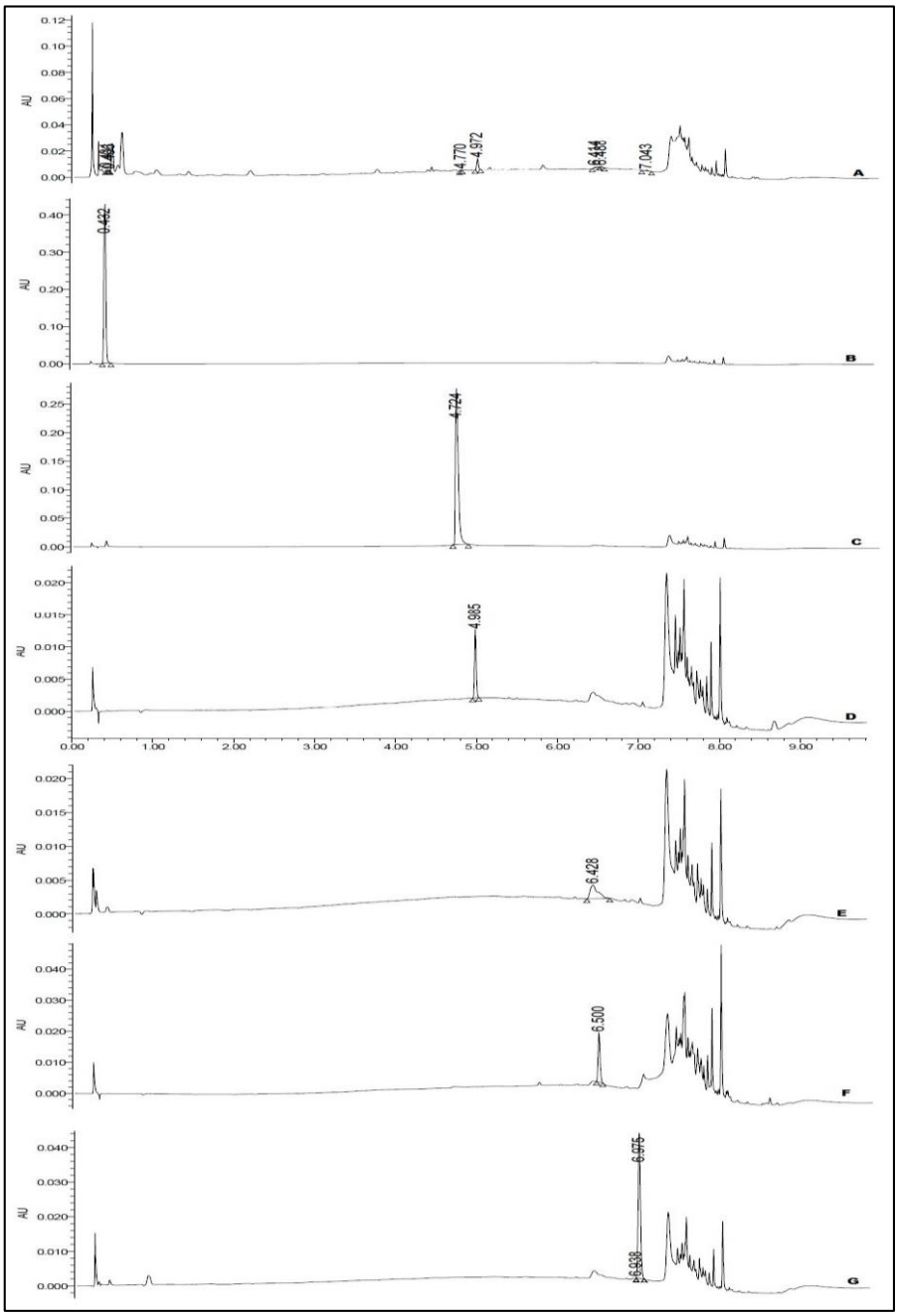

Fig 5A-F: Overlay spectra of HPLC A-Extract, B-Gallic acid, C—Ellagic acid D-Rutin, E-Catechin, F-Quercetin, G-Apigenin

4. Conclusion

Quality of herbal products can be considered only when they are characterized and authenticated. These studies can promote the quality of crude herbs and herbal products. *C. deciduas*, a xerophytic plant is widely available, inexpensive, literature also explains its therapeutic worth. Thus its quality control, safety and phytochemical profile can be a great contribution to the healthcare of society.

Declaration of Interest

Authors declare no conflict of interest.

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