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# PHYSICOCHEMICAL ANALYSIS AND HPTLC STUDIES OF *GOSSYPIUM HERBACEUM* LINN. (FLOWER)

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**Abstract**

*Gossypiumherbaceum* Linn. belongs to the family Malvaceae, is commonly known as Karpas or Cotton**.** The plantoccurs in Middle East countries,Central Asia and Western India. The area of its greatest variability is found in Baluchistan, Iran, Afghanistan and Russian Turkestan. The cultivated types are found in India, China and Middle East countries. *Gossypiumherbaceum* has been widely used in theproduction of food and medicine as well. The parts of the plant used in medicine are seeds, leaves, flowers, root and root bark. The plant possesses antifertility, antispermatogenic, antitumor, abortifacient, contraceptive, antidiabetic, antiulcer, antiviral and antibacterial activities. In the present paper, a detailed study on *Gossypiumherbaceum*(flower) based on its physico-chemical analysis and High Performance Thin Layer Chromatographic (HPTLC) studies were carried out to lay down the pharmacopoeial standards. The physico-chemical parameters such as loss on drying at 105$^{o}$C, solubility in water and alcohol, ash content and acid insoluble ash were determined by standard methods. A preliminary phytochemical study was carried out using different extractives of the plant material. HPTLC profile of the ethanol extract of the plant material was carried out in UV 254nm, UV 366nm and using vanillin- sulphuric acid as derivatisation reagent and the $R_f$ values were noted. All these parameters are helpful in identification and standardization of the flower of *Gossypiumherbaceum*.

**Key Words:** *Gossypiumherbaceum, P*hysico-chemical properties, HPTLC profile, Pharmacopoeial standards.

## Introduction

Indian System of Medicine deals with both preventive and curative aspects of life in a most comprehensive way and presents a close similarity to the WHO's concept of health propounded in the modern era. The efficacy and popularity of

any system of medicine depends on the quality of medicines used for the treatment. To assure the quality of medicines, standardization plays the vital role for producing effective drugs and is the need of the present hour.

In the present paper, a detailed study on *Gossypiumherbaceum*(flower*)* based on its physico-chemical analysis and High Performance Thin Layer Chromatographic (HPTLC) studies were carried out to lay down the pharmacopoeial standards. *Gossypiumherbaceum*Linn.belongs to the familyMalvaceae, commonly known as Karpas or Cotton[1]. The plantoccurs in Middle East countries,Central Asia and Western India. The area of its greatest variability is found in Baluchistan, Iran, Afghanistan and Russian Turkestan. The cultivated types are found in India, China and Middle East countries[2]. The vernacular names of the plant are Sanskrit-Tundakesi, Karpas, Anagnika; Hindi-Kapas; Kannada-Hati, Arale, Ambara, Karpasa; Bengali- Karpas, Tula; Gujarati- Vona, Rui; Telungu- Patti; Tamil- Paruthi; Malayalam- Karuparuthi[3].Unani-Pambadana; Arabic-Habbulqutn; English-Bona, Kapsia, Common cotton, Indian cotton, Levant cotton; Urudu-Pambadana, Habulqutn; Binaula, Kapas; Persian-Pambadana[4,5,6,7,8].

*Gossypiumherbaceum*plant is mentioned in indigenoussystems of medicine. It is an erect, shrubby, hairy plant, 2-8 feet high with thick woody stem and twigs and leaves sparsely hairy, rarely glabrous. The leaves are 5-7 lobed, lobes ovate, and rotund, only slightly constricted at base. Bracteoles with 6-8 serrated teeth on the margin, broadly triangular, usually broader than long. The flowers are large, yellow with purple center; calyx base is black with glandular dots and capsules ovate, pointed[4,5]. Presence of eye-rays on corolla is the typical character of *G.herbaceum*. The seeds after the removal of fuzz are dark brown or nearly black in colour, pointed ovoid in shape and vary in size from 5.0 to 20 mm in length. Flowering and fruiting time of the plant is January to April.

**Figure 1:***G.herbaceum* plant

**Figure 2:***G.herbaceum* flower

*Gossypiumherbaceum*has been widely used in theproduction of food and medicine as well. The parts of the plant used in medicine are seeds, leaves,flowers, root and root bark[4,5]. Cotton seeds are not only a valuable source of vitamins but an excellent pain reliever. It is useful as a nervine tonic in treating certain neurological conditions such as headache,

migraine, etc., and decoctions of the seed are given in dysentery and intermittent fever. The seeds and flowers in the form of poultice is applied to burns and scalds.[8,9] Gossypol, a phenolic compound present in the plant is used in treating endometriosis and uterine bleeding. Decoction of the flowers and seeds are anti-dote to *datura* poisoning. Flowers are useful in uterine discharge.[10] Flowers are liver stimulant and pleasant to mind. Syrup of the flower is useful in hypochondriasis on account of its stimulating and exhilarating effect. The aqueous and ethanolic extracts of flowers of *G.herbaceum* increases healing of gastric ulcer and possess potential antiulcer activity[11]. Seeds are also useful in epilepsy and also as an antidote to snake poison. Young fruit is given to correctthe dysentery.The cotton seed oil is useful to cure head ache[3].It is also useful in clearing the spots and freckles of the skin[8]. The taste of seeds is slightly bitter. The juice of the leaves is useful in dysentery. The leaves externally in the form of poultice hasten the maturation of boils and with oil they are applied as a plaster to gouty joints.[8] Root bark with leaves of *Bambusaarundinacea* (bans) is used for inducing abortion. Leaves of *G.herbaceum* and *Bambusaarundinacea*(*Bans*) are given orally to augment labour.Leaves and root of the plant are given orally in retention of placenta.[12]The root has emmenagogue property, useful in dysmenorrhoea and suppression of the menses produced by cold. It is used to enhance the first stage of labour. It is used for cough, asthma and sexual weakness[8]. The plant increases blood and urine. It is used in diseases of ear[3]. *Gossypiumherbaceum* possesses antifertility[13], antispermatogenic, antitumor[14], abortifacient, contraceptive[15], antidiabetic, antiulcer, antiviral[1] and antibacterial activities[16]. The plant has therapeutic properties like Madhurarasam,ushnaveeryam,vatha haram and lagu guna[3].

It is a known fact that the members of *Gossypium* genera contain Gossypol glands. Gossypol, a phenolic compound is found in gossypol glands. Presence of gossypol plays an important role in conferring resistance against pests. Gland density varies in different parts of plant. Similarly gossypol content also differs on different plant tissues. Besides Gossypol, seeds contain Quercetin, Betaine, Choline, Salicylic acid etc. Flowers contain a glucoside namely gossypetin. Bark contains starch, a chromogen, glucose, yellow resin, fixed oil, albinoids and lignin. Root bark contains yellow or colourless acid resin, dihydroxy benzoic acid and phenols[3].

## Materials and Methods

**Collection of the plant material:** Flowers of *Gossypiumherbaceum* Linn.were collected from Mettur Botanical Garden. The plant material was dried in shade, cut, crushed and kept in airtight bottle for experimental purpose.

**Physico-chemical parameters**

The physico-chemical analysis such as determination of ash value, acid insoluble ash, extractable matter in water and alcohol and loss on drying at 105°C were carried out by standard methods[17,18]. The information collected from these tests is useful for standardization.

**Preliminary phytochemical analysis**

For preliminary phytochemical studies, 5g powdered material was successively extracted using soxhlet apparatus with petroleum ether, chloroform, ethanol and water. The extracts were concentrated by distilling off the solvents under reduced pressure. The presences of different phyto-constituents were determined by standard procedure[19,20]. The qualitative chemical tests were carried out for the identification of the nature of different phyto-constituents present in the single drug.

**High performance thin layer chromatographic analysis (HPTLC)**

HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials and is the simplest separation technique today available to the analyst[21].

**Preparation of extract of the drug material for HPTLC analysis**

Extract of the plant material was prepared by boiling 1g of the drug in 10 ml ethanol. The filtrates were concentrated on a water bath to 1 ml. This extract was used for chromatographic studies[22].

**Development of high performance thin layer chromatographic (HPTLC) profile**

HPTLC is a micro analytical separation and determination method which has a wide application in herbal drug analysis. Alcohol extract of the plant material was spotted in the form of bands with Camagmicrolitre syringe on a precoated silica gel 60 $F_{254}$(Merck) plate with Automatic TLC Sampler 4 (ATS4). Mobile phase used was Toluene: Ethyl acetate (5:2). Linear ascending development was done in twin trough glass chamber saturated with mobile phase. The plate was air dried and kept under UV 254 nm and 366 nm, and derivatised using vanillin-sulphuric acid reagent and photodocumentation were done[10]. The plate was scanned in UV 254 nm, 366nm and in white light (575nm) using TLC Scanner 4 with winCATS software for interpretation of data.

## Results and Discussion

### Physico-chemical parameters

The physico-chemical parameters of the flower of *G. herbaceum* are given in Table 1, which are important diagnostic features of the plant.

**Table 1.Physico-chemical parameters of *Gossypium herbaceum* L.(Flower).**

| Sl.No. | Parameters | Mean* |
|---|---|---|
| 1. | Foreign Matter % | <2 |
| 2. | Loss on Drying at $105^0$C % | 11.37 |
| 3. | Total Ash Content % | 10.38 |
| 4. | Acid Insoluble Ash % | 0.48 |
| 5. | Water Soluble Extractive % | 16.20 |
| 6. | Alcohol Soluble Extractive % | 6.75 |
| 7. | Volatile oil % | Nil |

***The values represent the mean value obtained for three different samples.**

The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign matter such as metallic salts or silica. This value also gives an idea about the total inorganic content. The amount of acid-insoluble siliceous matter present was 0.48. The water soluble extractive was found to be 16.20; this value indicated the presence of sugar, acids and inorganic compounds present in the drug. The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids etc. The alcohol soluble extractive was found to be 6.75 which signify that the less amount of constituents of the drug was soluble in alcohol[17,18].

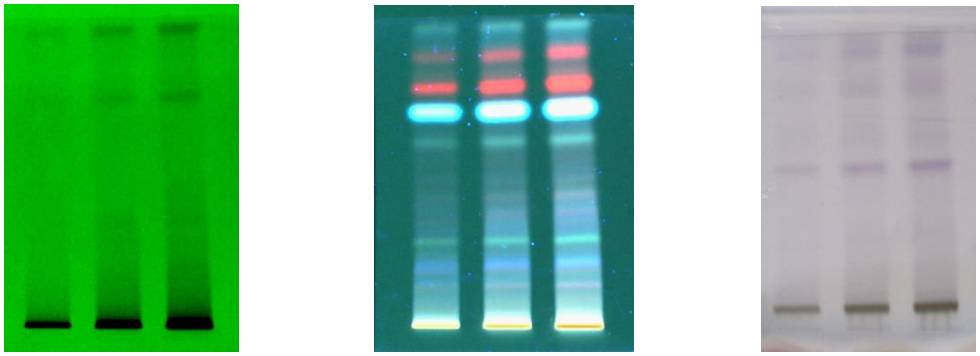
### Preliminary phytochemical analysis

The preliminary phytochemical investigations of the flower was performed which shows the presence of carbohydrates, flavonoids, tannins, steroids, terpenoids, saponins, resins, phenols and proteins which are the major secondary metabolites responsible for the potent therapeutic activity of the plant material.

### High performance thin layer chromatographic analysis (HPTLC)

The HPTLC fingerprinting patterns of ethanol extracts of the flower of *G.herbaceum* was developed at 254nm, 366nm and after derivatisation with vanillin – sulphuric acid at 575nm. The solvent system, Toluene: Ethyl acetate

(5:2)efficiently resolved the components present in the crude extract. HPTLC photo documentation profile of the ethanol extract of *G.herbaceum* at 254nm, 366nm and after derivatisation is given in Fig.3. On observation 4 bands were appeared under UV at 254nm with $R_f$ 0.21, 0.35, 0.76 and 0.98. TLC pattern at 366nm showed 7 bands at $R_f$value 0.02, 0.21, 0.43, 0.61, 0.71, 0.79 and 0.89. The 3D densitometric chromatogram of the alcohol extract of *G.herbaceum* (flower) at 575nm and the HPTLC fingerprinting photodocumentationare given in Figures 4 and Figure 5 respectively and the $R_f$value and percentage area of the peaks are shown in Table 2.



**Viewed underUV 254** **Viewed underUV 366DerivatisedinVanillin –Sulphuric acid and viewed in white light**

**Fig.3: HPTLC photo documentation profile of the ethanol extract of *G.herbaceum***

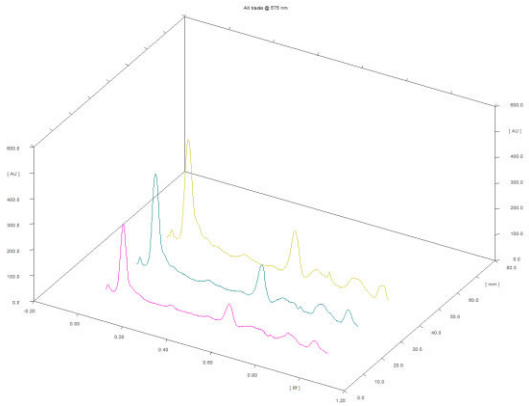


**Figure 4 : 3D densitometric chromatogram at 575nm of 10,15 and 20 µl of ethanol extract of *G.herbaceum***

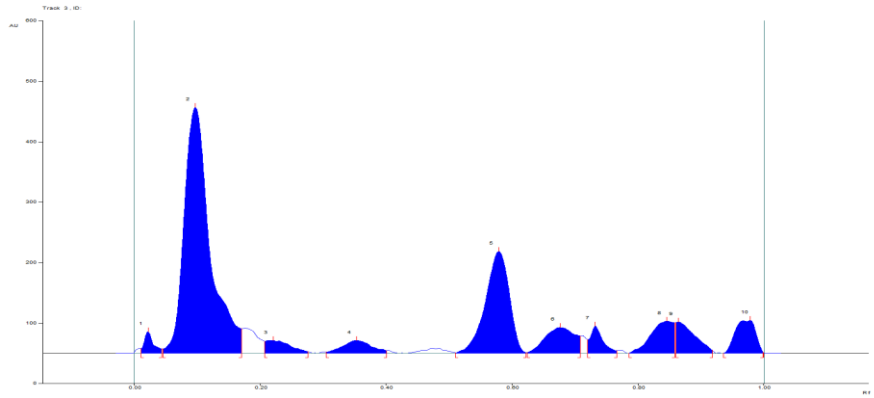


**Figure 5: HPTLC Finger print profile of ethanol extract of*G.herbaceum* at 575nm**

**Table-2: $R_f$ table of ethanol extract of *G.herbaceum* at 575nm.**

Track 3, ID: Gh alcohol extract

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 Rf | 8.0 AU | 0.02 Rf | 35.3 AU | 3.93 % | 0.04 Rf | 7.3 AU | 417.2 AU | 1.56 % |
| 2 | 0.05 Rf | 7.3 AU | 0.10 Rf | 406.6 AU | 45.21 % | 0.17 Rf | 40.7 AU | 13229.6 AU | 49.63 % |
| 3 | 0.21 Rf | 19.7 AU | 0.22 Rf | 21.2 AU | 2.36 % | 0.28 Rf | 2.6 AU | 666.6 AU | 2.50 % |
| 4 | 0.31 Rf | 2.0 AU | 0.35 Rf | 21.1 AU | 2.35 % | 0.40 Rf | 4.5 AU | 780.0 AU | 2.93 % |
| 5 | 0.51 Rf | 1.0 AU | 0.58 Rf | 168.7 AU | 18.76 % | 0.62 Rf | 1.0 AU | 5011.0 AU | 18.80 % |
| 6 | 0.62 Rf | 1.2 AU | 0.68 Rf | 42.5 AU | 4.73 % | 0.71 Rf | 28.6 AU | 1614.4 AU | 6.06 % |
| 7 | 0.72 Rf | 22.7 AU | 0.73 Rf | 44.9 AU | 4.99 % | 0.77 Rf | 4.3 AU | 715.2 AU | 2.68 % |
| 8 | 0.79 Rf | 0.1 AU | 0.85 Rf | 53.1 AU | 5.90 % | 0.86 Rf | 50.5 AU | 1547.3 AU | 5.80 % |
| 9 | 0.86 Rf | 50.6 AU | 0.87 Rf | 51.6 AU | 5.74 % | 0.92 Rf | 4.8 AU | 1252.8 AU | 4.70 % |
| 10 | 0.94 Rf | 0.2 AU | 0.98 Rf | 54.3 AU | 6.04 % | 1.00 Rf | 2.3 AU | 1423.6 AU | 5.34 % |

HPTLC finger print profile of the ethanol extract of *G. herbaceum* (flower) was developed after derivatising in vanillin-sulphuric acid and scanned at 575nm. There were ten peaks observed of $R_f$values at 0.2, 0.10, 0.22, 0.35, 0.58, 0.68, 0.73, 0.85, 0.87, 0.96. Out of these, two peaks ($R_f$ 0.10 and 0.58) were found to be more prominent and this implies that these chemical constituents were present in significant quantity in the crude extract.

**Conclusion**

The different physico-chemical parameters, the preliminary phytochemical analysis and the developed HPTLC chromatogram obtained from this study help in identification and standardization of the drug. Standardization is essential measure for quality, purity and sample identification. HPTLC fingerprinting profile is a very important parameter of standardization for the proper identification of medicinal plants. The HPTLC fingerprinting profile developed along with the physico- chemical parameters can be used as a diagnostic tool to identity and to determine the quality and purity of the flower of *Gossypiumherbaceum* .

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