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# PHARMACOGNOSTICAL STUDY AND ESTABLISHMENT OF QUALITY PARAMETERS OF WHOLE PLANT OF *CRESSA CRETICA* LINN

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**ABSTRACT**

*With the emerging worldwide interest in adopting and studying traditional systems of medication and exploiting their potential based on different health care systems, the evaluation of the rich heritage of traditional medicine is essential. From the innumerable plants being researched since time immemorial, Cressa cretica is an important one. To evaluate the diagnostic Pharmacognostical characters of Cressa cretica along with their physico-chemical parameters, fluorosence analysis and TLC profile. The Pharmacognostical characters were determined in terms of macroscopy, microscopy, powder microscopy, leaf constant, fluorescence analysis and preliminary phytochemical analysis. The findings of macroscopy revealed that leaves ovate or elliptic-to scale like, flowers solitary, axillary, sessile or on short peduncles, fruits capsular, ovoid, unilocular, Seed one seeded, glabrous and smooth & stem first erect and then become decumbent with horizontal Roots. Transverse section of stem depicted the single celled epidermis, hypodermises, cortex, parenchymatous cells, lignified xylem. Transverse section of root septate hairs, epidermis, arenchyma, starch grains, Cortex, spherical vesicles. Transverse section of leaf palisade cells, trichomes, paracytic and anomocytic stomata. Preliminary phytochemical screening exhibited the presence of various phytochemical groups like Alkaloids, Carbohydrate, Flavonoids, Fixed Oils & Fats, Glycosides, Steroids, Tannins & phenolic compound. Further the leaf constant, powder microscopy, fluorescence characterstics & TLC profile indicating results from this investigation. Various Pharmacognostical and physico-chemical parameters have pivotal roles in identification, authentication and establishment of quality parameters of the species.*

**Key words:** *Cressa cretica*, Pharmacognostical characters, Physico-chemical parameters, Fluorosence analysis, Phytochemical groups, Quality parameters

## INTRODUCTION

World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species [1]. According to the WHO more than 80% of the world's population realize on traditional herbal medicine for their primary health care [2]. Although herbal medicine has existed since the dawn of time, our knowledge of how plants actually affect human physiology remains largely unexplored. Numbers of plants are claiming various medicinal uses and many researches are going on in this view. India is one among the 25 hotspots of the richest and highly endangered eco-regions of the world [3].

Traditional medicine is defined as the sum total of the knowledge skills and practices, based on the weather applicable or not, used in diagnosis, preventive and elimination of physical, mental and social imbalances and relying exclusively on particular experience and observation handed down from generation to generation, whether verbally or in writing. In another way, a traditional medicine may also be considered as a solid combination of dynamic medical knowledge and ancestral experience [4].

Prior to any research on herbal medication, it is very pivotal to estimate and analyze the standardization of any medicinal plant. *Cressa cretica* is a potentially active plant.

*Cressa cretica* (Linn) belonging to family Convolvulaceae, commonly known as Rudravanti is an erect, small, dwarf shrub [5]. It is a remarkable salt tolerant plant, common in coastal areas [6] usually occurring in mono specific stands along the landward edge of marshes [7].

The plant is traditionally used in Bahrain as expectorant and antibilious agent and also reported to be antitubercular, antibacterial, antiviral, antidiabetic, alterative, anthelmintic, stomachic, tonic, aphrodisiac, enriches the blood and is useful in constipation, leprosy, asthma and urinary discharges [8, 9,10,11].

Phytoconstituents isolated from this species include: coumaranochromone glycoside as cresoside, acyclic terpenic compounds namely cressanyl ester A, B, C, D, E, F and G, and cressatriterpenic acid, flavonoids which were identified as quercetin, quercetin-3-O-glucoside, kampferol-3-O- glucoside, kampferol-3-O-rhamnoglucoside and rutin [5] moderate amount of terpenes and tannins [12].

The present investigation used different pharmacognostic and phytochemical parameters to supplement the identification and standardization information.

## MATERIALS & METHOD

***Plant materials***

The whole plant of *Cressa cretica* L. Convolvulaceae was collected for the research work from Andra Pradesh and authenticated by Dr. K. Madhava Chetty, plant taxonomist (IAAT, 357), Shri Venkateswara University, Andra Pradesh, India. A voucher specimen (Specimen no. 1229) and crude drug sample was preserved in the department of Pharmacognosy, S. D. College of Pharmacy & Vocational Studies, Muzaffarnagar.

For Pharmacognostical studies plant material was stored in a solution containing 5 ml formaldehyde, 5 ml acetic acid and 90 ml 70% alcohol and rest of remaining plant material was further size reduced and stored in an air tight container. Reduced form of drug was employed for determination of physicochemical parameters like moisture content, ash values, swelling index, foaming index, foreign organic matter, extractive values, qualitative and fluorescence analysis.

***Macroscopic & microscopic analysis***

The Organoleptic characteristics such as colour, odour, taste, texture, shape, size, of *C. cretica* were determined. The macroscopy, microscopy and powder characteristics of plant were studied according to the standard methods [13, 14]. Leaf constants viz. stomatal index, stomatal number, Vein-Islet and Vein-Termination number were also studied according to the

standard procedures [13]. Stomatal indices of both adaxial and abaxial epidermis in 1 $mm^2$ leaf surface was determined and recorded by using camera Lucida and stage micrometer.

Free hand sections were taken for microscopical evaluation and studies were conducted as per standard methods [13], Iodine, Potassium Iodide (IKI) & Aniline Blue in lactophenol was used for differential staining along with Phloroglucinol & HCl [15, 16]. Concentrated nitric acid (50%) with a pinch of potassium chlorate was used as the macerating fluid.

Powdered drug was used for powder microscopy. Photomicrographs were obtained by compound binocular microscope OLYMPUS BX41 and photomicrography was done using Olympus C7070 camera.

***Physico-chemical analysis***

Air dried plant drug was used for the quantitative determination of moisture content, ash values, swelling index, foaming index, foreign organic matter, extractive values via standard methods [13,14,17-19].

The total ash value for a crude drug is not always reliable, since there is a possibility of presence of non-physiological substances such as earthy matters. So, the parameters such as acid insoluble, water soluble and sulphated ash values were performed. Extractive values with petroleum ether, chloroform, ethyl acetate, ethanol and water were also determined.

The fluorescence analysis is a tool for the determination of constituents in the plant that gives a definite idea of the chemical nature. Fluorescence analysis of crude powdered drug and various extracts was carried out by standard method [20] & observation in colour was made in visible light, U.V light of short (254nm) and long wavelength (365nm) under U.V chamber [21].

***Preliminary phytochemical analysis***

Preliminary phytochemical screening was performed by using standard procedures [13, 14 & 22]. The extracts obtained from different solvents were subjected to identification tests for the detection of different phytoconstituents through organic and inorganic elements analysis via the method of Khandelwal & N Raman. TLC Analysis was also performed by using standard procedures [18, 23]. The study of various extracts to obtain the number of constituents present in each extract.

## RESULT

***Macroscopic characters***

It referred to the evaluation of a drug by colour, odour, size, shape, taste and special features such as: touch, fracture, texture etc. which were evaluated with the help of sense organs (Figure 1& Table 1).


**Figure 1:** Dimensions, leaves and flowers of Cressa cretica.

**Table 1:** Organoleptic evaluation of *Cressa cretica* (whole plant)

| S.No | Character | Observation |
|---|---|---|
| **1** | **Colour** | Dull green |
| **2** | **Odour** | Characteristic pleasant |
| **3** | **Taste** | Unpleasant or sour |
| **4** | **Texture** | Coarse |
| **5** | **Shape** | ovate or elliptic-to scale like, sessile (leaves)<br>solitary, axillary, sessile or on short peduncles, bracteate, ovate to obovate imbricate (flowers)<br>capsular, ovoid, unilocular(fruits)<br>one seeded, glabrous and smooth(Seed)<br>at first erect and then become decumbent<br>cylindrical to oval (stem )<br>horizontal (Root) |
| **6** | **Size** | 18 cm in height<br>1-12 mm long (leaves)<br>5-8 mm long(flowers)<br>3-4 mm long (Seed)<br>2-4-valved (fruits) |

***Microscopic characters***

**Transverse Section (Stem) Examination:** A suitably designed transverse section of stem was studied & different tissue organizations were viewed at 10X & 40X (Figure 2).

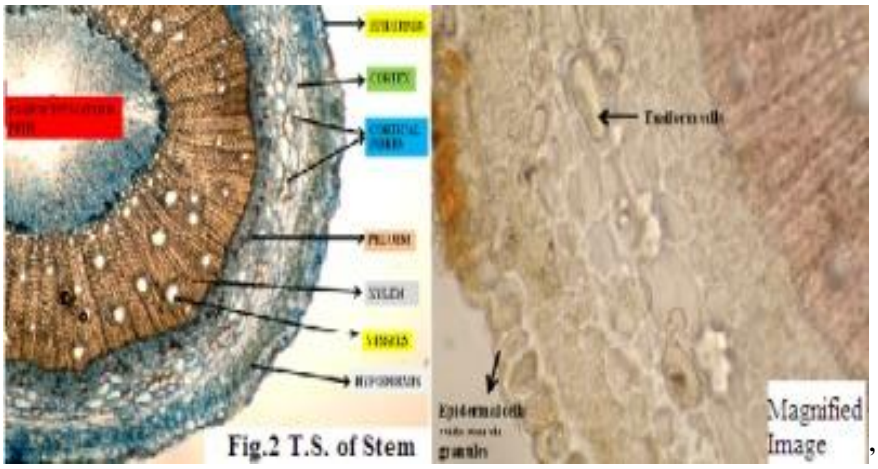


**Figure 2:** TS of stem and magnified image.

Transverse section of the stem of *Cressa cretica* (Fig.2) depicted the single celled epidermis consisting of oval or barrel shaped cells measuring 14-21µ which was cellulosic in nature and took a blue stain on treatment with $I_2$+KI & dilute $H_2SO_4$. Epidermis

was followed by 1-2 celled hypo dermises almost similar to that of epidermis. Next to hypodermis was cortex 2-3 layered which consisted of thin walled, loosely arranged large parenchymatous cells which was polygonal, tangentially elongated and fusiform in shape. In the cortex lied the bundle of cortical fibers consisting of 3-5 fibers and each measuring 10-16 µ in diameter.

Next to cortex lied the phloem region which took deep blue stains with $I_2$+KI and aniline blue thus depicting the callsoe plugs of sieve tubes. Next to phloem region was the lignified xylem region consisting of vessels scattered throughout the ground tissue with protoxylem towards the pith and metaxylem towards the periphery. The centrally located pith consisted of thin walled parenchymatous cells. The epidermal cells contained starch grains which were depicted in (Figure 2).

***Transverse Section of root***

T.S of the root consisted of septate hairs covering the entire epidermal region, epidermis contained dark brown coloured 2-3 layered cork cells followed by the cortical region comprising of large number of spherical or sub spherical vesicles filled with starch grains which took blue colour on treatment with I2+KI and aniline blue. Cortex also contained arenchyma having large intercellular spaces which was commonly seen in plants growing in water logged soil and it occurred due to deficiency of oxygen which caused the production of ethylene leading to the programmed cell death of some tissues resulting in creation of large intercellular spaces. Next to cortex region is the light brown coloured phloem region which stains light blue with I, KI and aniline blue. Next to Phloem region is xylem consisting of xylem vessels scattered in the entire lignified ground tissue and biserate medullary rays extending from the pith up to the phloem region (Figure 3).

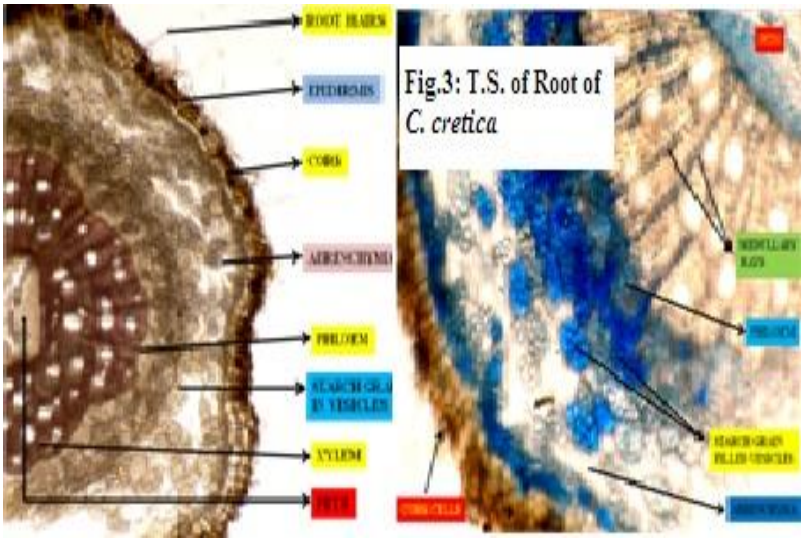


**Figure 3:** TS of root of C. cretica.

***Transverse section of Leaf***

Microscopical study to the leaf of *Cressa cretica* revealed its isobilateral nature with palisade cells on both upper and lower surfaces and covering trichomes on the upper surface of the leaf along with paracytic and anomocytic stomata which was found evenly dispersed in the measophyll of the leaf (Figure 4)


**Figure 4:** TS of leaves of Cressa cretica

***Leaf constants***

Such determinations were performed as per method available in the literature & the obtained were as shown in (Table 2)

**Table 2:** Leaf constants of *Cressa cretica*

| S.No. | PARAMETERS | RESULT |
|---|---|---|
| **1.** | Stomatal number | Upper surface – 0.19<br>Lower surface – 0.30 |
| **2.** | Stomatal index | Upper surface – 17.82<br>Lower surface – 26.11 |
| **3.** | Number of Vein-Islet | 13 |
| **4.** | Number of Vein-Termination | 9 |

**Powder Microscopy of *Cressa cretica***

Powder study of the stem and root reveals the fibers [(Fig-5(I)] which are spindle shaped with a wide lumen and oblique pits and measures 270-350µ in length and 10-16µ in diameter. Some trachedial fiber measuring 200 µ-300 µ are also observed [(Fig-5(II)].Vessels with scalariform and simple pits are also observed [(Figure-5 (III & IV)].

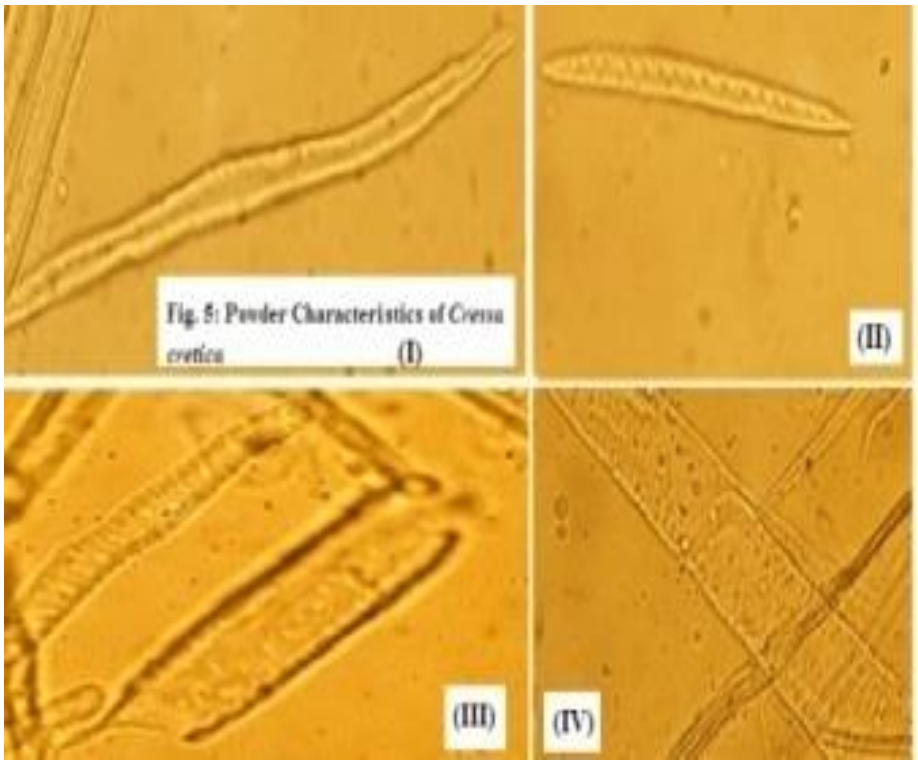


**Figure 5:** Powder characteristics of Cressa cretica

***Physico-chemical analysis***

Air dried plant drug was used for quantitative determination of physico-chemical parameters such as total ash, acid insoluble ash, water soluble ash and sulphated ash are shown in Table 3. Sulphated ash value (17.5%) was lower than the total ash value (22.2%). The acid insoluble and water soluble ash values were 15.1% and 12.3%, respectively. Further, the results also showed that moisture content, foreign organic content, foaming index and swelling index were found to be 1.52%, 0.30%, no foreign content, less than 100 and 0.4cm respectively (Table 4). The extractive values for various solvents such as petroleum ether, chloroform, ethyl acetate, ethanol, chloroform water and hydro alcoholic were found to be 1.2%, 3.1%, 2%, 2.5%, 18% and 12.8% respectively (Table 5). All these parameters were recorded for five times and written as mean ± standard error of mean (SEM).

**Table 3:** Ash values of *C. cretica*

| S. No. | Parameters | % Yield (w/w) |
|---|---|---|
| **1.** | Total ash values | 22.2 |
| **2.** | Acid-insoluble ash values | 15.1 |

| | | |
|---|---|---|
| **3.** | Water-soluble ash values | 12.3 |
| **4.** | Sulphated ash values | 17.5 |

**Table 4:** Moisture content, Foreign Organic Matter, Foaming Index and Swelling Index of *C. cretica*

| S. No. | Parameter | % Yield (w/w) |
|---|---|---|
| **1.** | Moisture Content (Loss on Dying) | 1.52 |
| **2.** | Foreign Organic Matter | NIL |
| **3.** | Foaming Index | Less than 100 |
| **4.** | Swelling Factor | 0.4cm |

**Table 5:** Extractive values of *Cressa cretica*

Extractive values for the plant drug sample were determined using different solvents i.e. petroleum ether, chloroform, ethyl acetate, ethanol, Chloroform water and Hydro alcoholic.

| S. No. | Extracts/Solvent | % Yield (w/w) |
|---|---|---|
| **1.** | **Petroleum ether** | 1.2 %W/W |
| **2.** | **Chloroform** | 3.1 %W/W |
| **3.** | **Ethyl acetate** | 2.0 %W/W |
| **4.** | **Ethanol** | 2.5 %W/W |
| **5.** | **Chloroform water** | 18%W/W |
| **6.** | **Hydro alcoholic** | 12.6 %W/W |

## Fluorescence Analysis

The fluorescence studies of crude powdered drugs and various extracts were determined under day/visible light and UV radiation of long and short wavelengths. When the powdered drug and extracts were treated with different reagents and observed under UV and ordinary light, they emitted various fluorescence or colour radiations. The colour change for the crude powder and individual extract were distinctive and reproducible revealing the solvent properties to the phytoconstituents and data is presented in the (Table 6).

**Table 6:** Fluorescence Analysis of whole plant powder of *Cressa cretica*

| S. No. | Treatment /Material | Day /Visible Light | Observation under U.V. Light (wavelength) | |
|---|---|---|---|---|
| | | | At Short(254nm) | At Long (365nm) |
| **1** | Drug Powder | Light brown | Dark brown | Dark brown |
| **2** | Powder treated with nitrocellulose in amyl acetate | Green | Fluorescent green | Fluorescent yellow |
| **3** | Drug Powder rubbed on filter paper | Dark brown | Dark brown | Dark brown |
| **4** | Powder treated with Methanolic 1 N NaOH | Brown | Creamish yellow | Light yellow |
| **5** | Powder treated with 1 N NaOH in Water | Dark brown | Brown | White |
| | Powder treated with 1N NaOH in nitrocellulose in amyl acetate | Fluorescent green | Fluorescent green | Fluorescent yellow |
| **6** | Powder treated with 1N HCl | Light brown | Black | White |
| **7** | Powder treated with 50% KOH | Light green | Dark Fluorescent green | Fluorescent yellow |
| **8** | Powder treated with 50% $HNO_3$ | Yellowish brown | Dark yellow | Black |
| **9** | Powder treated with 50% $H_2SO_4$ | Brown | Brown | Brown |
| **10** | Powder treated with acetic acid | Light green | Fluorescent yellow | Green |
| **11** | Ethanolic Extract | Brown | Dark brown | Black |
| **12** | Water Extract | Brown | Black | Light brown |
| **13** | Chloroform Extract | Brown | Brown | Light brown |
| **14** | Petroleum ether Extract | Light brown | Light brown | Off white |
| **15** | Dragendorff's reagent | Brown | Brownish black | Dark brown |
| **16** | Picric acid | Light brown | Green | Dark brown |
| **17** | $FeCl_3$ | Dark brown | Dark green | Brownish-black |

***Preliminary phytochemical screening***

The various extracts i.e. petroleum ether, chloroform, ethyl acetate, ethanolic and aqueous extracts were subjected to preliminary phytochemical screening through qualitative chemical tests.

The phytochemical profiling of the plant revealed the presence of alkaloid, flavonoids, proteins, amino acids, phenols, tannins, gum & mucilage, glycosides and phytosterol which serves as an important tool for the quality assurance of plants for further studies as organic phytoconstituents (Table 7). Qualitative analysis of various inorganic elements revealed the presence of iron, sulphates, phosphates and chlorides (Table 8).

***Detection of Organic Constituents***

**Table 7:** Phytochemical tests of *Cressa cretica*

| S. No. | Test Name | Name of the Extract | | | | |
|---|---|---|---|---|---|---|
| | | **Petroleum Ether** | **Chloroform** | **Ethyl acetate** | **Methanol** | **Aqueous** |
| **1.** | Alkaloids | - | - | - | + | + |
| **2.** | Carbohydrate | - | - | - | + | + |
| **3.** | Flavonoids | + | + | + | + | + |
| **4.** | Fixed Oils & Fats | - | + | + | + | - |
| **5.** | Glycosides | + | + | + | + | + |
| **6.** | Protein | - | - | - | - | + |
| **7.** | Saponin | - | - | - | - | - |
| **8.** | Steroids | - | - | - | + | + |
| **9.** | Tannins & phenolic compound | + | + | + | + | + |

## Table 8: Inorganic elements tests of Cressa cretica

| S.No | Tests | Filtrate | |
|---|---|---|---|
| | | **Hydrochloric acid** | **Nitric acid** |
| **1** | Calcium | - | - |
| **2** | Magnesium | - | - |
| **3** | Potassium | - | - |
| **4** | Iron | + | + |
| **5** | Sulphate | + | + |
| **6** | Phosphate | + | - |
| **7** | Chloride | + Silver nitrate) | + (Silver nitrate) |
| | | + (Lead acetate) | + (Lead acetate) |

***TLC Analysis***

The suspension of silica gel-G in distilled water was prepared and coated over the TLC plates. Further the plates were air dried and then activated in an oven at 110°C for 1 hour and allowed to cool & protected from moisture. The extracts being examined were applied in the form of circular spots, or in the form of bands. The developed spots were visualized by using detecting agent like anisaldehyde and Iodine and or by UV chamber at short wavelength (254 nm) and long wavelength (365 nm).The distance of each spot from the point of its application was measured and Rf values were calculated by the following formula:

**Table 9:** Thin layer chromatography of petroleum ether extract

| Condition | Petroleum Extract | |
|---|---|---|
| | **No. of Spot** | **$R_f$ values** |
| **Iodine** | 7 | 0.10, 0.27, 0.40, 0.65, 0.72, 0.80, 0.89 |
| **Short wave length (254nm)** | 4 | 0.43, 0.65, 0.74, 0.78 |
| **Long wave length (365nm)** | 4 | 0.43, 0.59, 0.71, 0.78 |
| **Anisaldehyde in $H_2SO_4$ (spraying reagent)** | 7 | 0.10, 0.27, 0.40, 0.65, 0.72, 0.80, 0.89 |

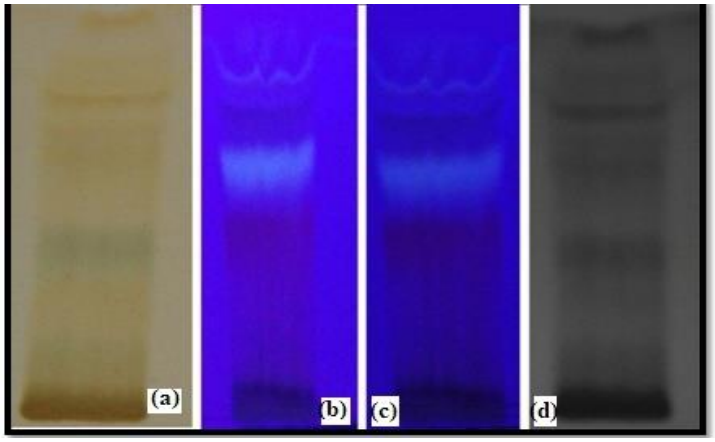


**Figure 6:** TLC of Petroleum ether extract (a) Iodine (b) Short UV (λ), (c) Long UV (λ), (d) Anisaldehyde in $H_2SO_4$

**Table 10:** TLC Analysis of Chloroform extract of whole plant of *Cressa cretica*

| Condition | Chloroform | |
|---|---|---|
| | **No of Spot** | **$R_f$ values** |
| **Iodine** | 7 | 0.13, 0.35, 0.41, 0.62, 0.73, 0.82, 1.17 |
| **Short wave length (254nm)** | 5 | 0.35, 0.82, 0.88, 0.94, 0.98 |

| | | |
|---|---|---|
| **Long wave length (365nm)** | 5 | 0.35, 0.82, 0.88, 0.94, 0.98 |
| **Anisaldehyde in $H_2SO_4$ (spraying reagent)** | 7 | 0.13, 0.35, 0.41, 0.62, 0.73, 0.82, 1.17 |

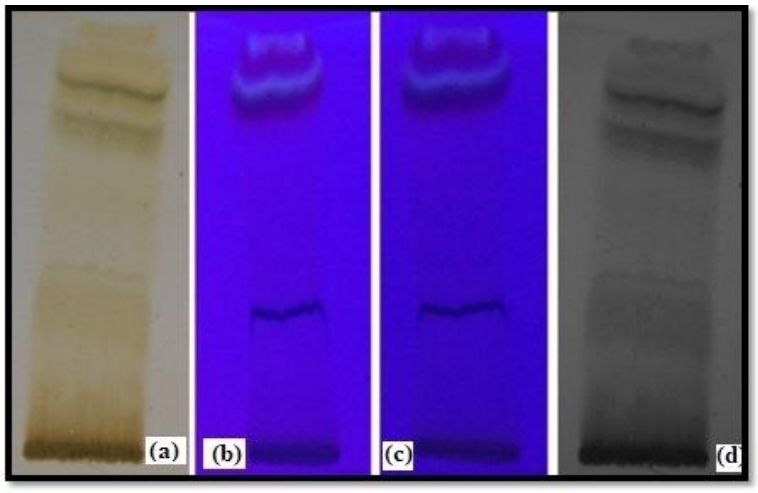


**Figure 7:** TLC of Chloroform extract (a) Iodine (b) Short UV (λ), (c) Long UV (λ) (d) Anisaldehyde in $H_2SO_4$

**Table 8:** TLC Analysis of Ethyl acetate extract of whole plant of *Cressa cretica*

| **Condition** | **Ethyl acetate** | |
|---|---|---|
| | **No of Spot** | **$R_f$ values** |
| **Iodine** | 7 | 0.14, 0.31,0.41,0.59,0.68,0.77,0.92 |
| **Short wave length (254nm)** | 8 | 0.89, 0.23, 0.34, 0.47, 0.56, 0.70, 0.77, 0.79 |
| **Long wave length (365nm)** | 8 | 0.89, 0.23, 0.34, 0.47, 0.56, 0.70, 0.77, 0.79 |
| **Anisaldehyde in $H_2SO_4$ (spraying reagent)** | 7 | 0.14, 0.31, 0.41, 0.59, 0.68, 0.77, 0.92 |

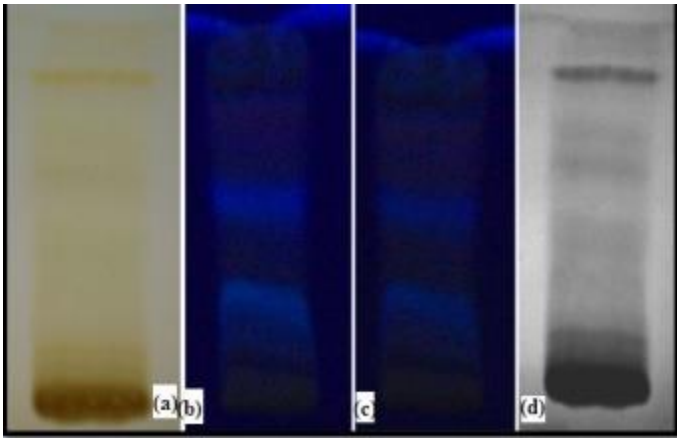


**Figure 8:** TLC of Ethyl acetate extract (a) Iodine (b) Short UV (λ), (c) Long UV(λ) (d) After spraying Anisaldehyde in $H_2SO_4$

**Table 12:** TLC Analysis of ethanolic extract of whole plant of *Cressa cretica.I*

| **Condition** | **Ethanolic** | |
|---|---|---|
| | **No of Spot** | **$R_f$ values** |
| **Iodine** | 4 | 0.29, 0.33, 0.59, 0.69 |
| **Short wave length (254nm)** | 3 | 0.48, 0.58, 0.61 |
| **Long wave length (365nm)** | 1 | 0.51 |
| **Anisaldehyde in $H_2SO_4$ (After spraying)** | 4 | 0.29, 0.33, 0.61, 0.72 |

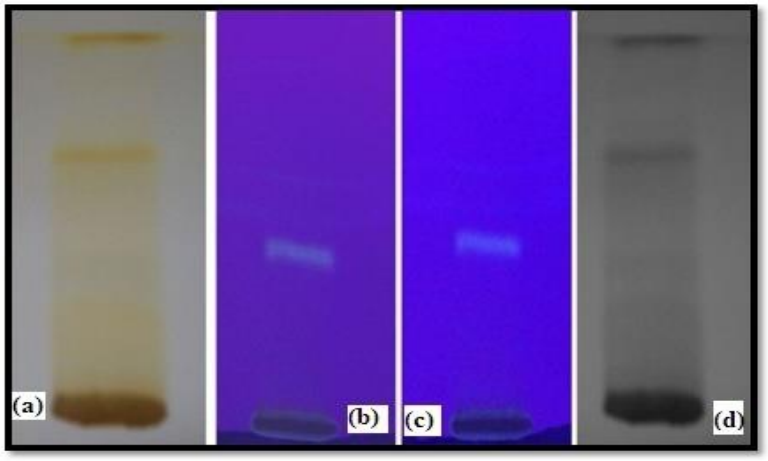


**Figure 9:** TLC of ethanolic extract (a) Iodine (b) Short UV (λ), (c) Long UV (λ) (d) After spraying Anisaldehyde in $H_2SO_4$

**Table 13:** TLC Analysis of aqueous extract of whole plant of *Cressa cretica*

| Condition | Water | |
|---|---|---|
| | No. of Spot | $R_f$ values |
| **Iodine** | 3 | 0.32, 0.66, 0.83, 0.93 |
| **Short wave length (254nm)** | 1 | 0.79 |
| **Long wave length (365nm)** | 1 | 0.79 |
| **Anisaldehyde in $H_2SO_4$ (After spraying)** | 4 | 0.32, 0.66, 0.83, 0.93 |

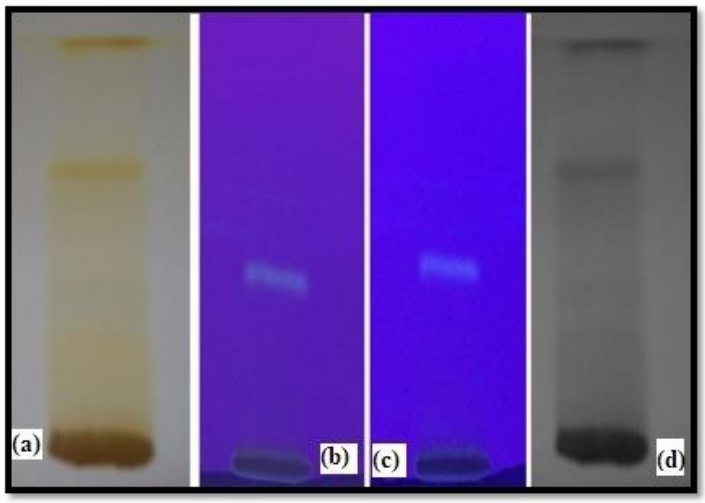


**Figure 10:** TLC of Aqueous extract (a) Iodine (b) Short UV (λ), (c) Long UV (λ) (d) Anisaldehyde in $H_2SO_4$

## CONCLUSION

*Cressa cretica* Linn, Convolvulaceae, is a remarkable salt tolerant plant and it is used for alterative, anthelmintic, stomachic, tonic, and aphrodisiac purposes, enriches the blood and is useful in constipation, leprosy, asthma and urinary discharges. The literature revealed that the plant consisted of various traditional and medicinal properties. The present study aimed to investigate & establish various pharmacognostical and physico-chemical parameters have pivotal roles in identification, authentication and establishment of quality parameters of the plant of *Cressa cretica.*

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