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CHROMATOGRAPHIC AND SPECTROSCOPIC STUDIES IN *Caesalpinia Crista* LINN.

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Key words : Chroomatographic, spectroscopic, *Caesalpinca crista*.

Seed extract of *Caesalpinia crista* Linn. Syn. *C. bonducella* showed variation in phytochemical attributes at intraspecific level. H_2SO_4 treated extract showed absorbance between 340 nm and 520 nm with λ-max at 380 nm. Development of straw orange colour after H_2SO_4 treatment indicated the presence of triterpene with cyclopentane perhydrophenanthrene ring system in the seed. Paper chromatography in acetone-butanol-water (1:2:10) also showed intraspecific variation. Intraspecific chemical variation may be exploited in terms of medicinal application of the plant seed.

INTRODUCTION

Caesalpinia crista Linn, is a large spinous shrub of the family Caesalpiniaceae growing wild alongside railway tracks and village shrubberies. The plant is well reputed as a source of medicines in Ayurveda and Unani systems of medicare. Seed of the plant is known to be styptic, antiperiodic, and anthelmintic, anti-inflamatory useful in colic malaria, hydrocele, skin disease and leprosy (Kritikar and Basu, 1935). An ayurvedic preparation known as 'bisan-jwaraghni-vati' from the seeds is prescribed as anthelmintic, anti-arthritis, anti-periodic, anti-pyretic, emmenagogue, expectorant, febrifuge and tonic (Dey, 1980).

The present paper discusses findings of chromatographic and spectral analyses of the aqueous seed extract.

MATERIALS AND METHOD

It was found that seeds of *Caesalpinia crista* are extracted by better in water than in acetic acid or ethyl alcohol or any other organic solvent for diagnostic purpose. When seeds are soaked in water for a period more than 24 hours, possibly most of the constituents of the seed percolate and seeds get choked. This is evident by the fact that Water extract presents high turbidity and foetid smell while the choked seeds do not yield any further percolation and they become smell-less.

For chromatography, seeds were extracted in distilled water for 24 hours by putting three rasped seeds in 15 ml of water. The extract was concentrated and dried in hot oven and spotted on a 24 x 24 cm Whatman paper number one. The spot was allowed to dry and a fresh spot was made on it. The process was repeated five times. Different solvents were tried including BAW (Butanol : Acetic Acid : Water as 4:1:5), forestal (cone HCL : Acetic Acid : water in 3:30:10 ratio), hexane - acetone (4:1), wateracetone (1:1), etc.

Saponins are known to be separated on PC with solvent Butanol saturated in water, while sapogenins are known to be separated better in the solvent hexane-acetone (Harborne, 1973). In the present study, best result was obtained in acetone: butanol : water : (1:2:10), in which three or four compounds were separated. The chromogenic reagent found as most effective was 50% H_2SO_4.

For spectrophotometry, visible light spectrophotometer 106 (Systronics) was used. Water extract was made by soaking 5 gm of seed in 20 ml of distilled water for 36 hours. 5 ml of this solution was taken as mother solution. For test solution, it was further diluted by mixing 4.5 ml of distilled water in 0.5 ml of mother solution. This test solution was taken for spectral characteristics against the blank solution of 5 ml distilled water. Optical density (absorbance) of the test solution in each case was measured at each wavelength between 340 and 930 nm of wavelength at 5 nm intervals. Above 610 nm, cut-off filter was used.

Graph was plotted for wavelength on X-axis against optical density on Y-axis.

RESULTS AND DISCUSSION

Seed extracts were prepared from seed of six randomly selected populations out of the 15 populations investigated. Seeds were collected from Junagadh (Gujrat) population (P_{15}), Gaya (Bihar) population (P_{14}), Arrah (Bihar) population (P_{12}), Patna. (Bihar) population (PII) one Sonepur (Bihar) population (P_9) and one Hajipur (Bihar) population (P_3). In all these cases, two types of spectral studies were made-one without putting H_2SO_4 to develop colour in the extract and one after putting H_2SO_4 to develop straw colour in the extract. Table-1 presents transmittance and optical density of test solution in relation to wavelengths in case of seed extracts untreated by H_2SO_4. Table-2 presents the same for H_2SO_4 treated extracts. Graph-1 presents plotting of absorbance against wavelength for seed extracts of four places treated by H_2SO_4.

It is known that spectral measurements are made in the range of 200-400 nm of wavelengths for colourless compounds. For coloured compounds, the range is usually between 200-700 nm. H_2SO_4 has been found to give straw orange colour to the seed extract. H_2SO_4 is known to give orange colour to phytosterols especially estrogen (Harborne, 1973). Therefore, presence of the triterpene with cyclopentane perhydrophenanthrene ring system is indicated in the seed of the plant. All the H_2SO_4 treated extracts showed absorbance between 340-520 nm with λ-max at 380 nm. Absorbance was also found after 900 nm. Between 520 and 900 nm, transmittance was mostly 100%, i.e., no absorbance took place (Table-2).

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As Table-2 shows Junagardh (Gujrat) extract is seemingly different from others in absorbance. Though λ-max is the same as 380 nm, the distinctiveness lies in the fact that transmittance is greater between 420 nm and 500 nm. It is 86.1 while in other cases it is lower. In case of Hajipur (Bihar) and Patna (Bihar) extracts which were identical, good absorbance took place also at 540 nm.

At higher wavelengths, Hajipur (Bihar) Patna (Bihar) extracts showed slight absorbance also at 920 nm. In this respect, Sonepur (Bihar) extract was more significant in showing absorbance at 900 nm itself. In case of Junagardh (Gujrat) extract, very little absorbance was found at 940 nm which seemed to progress thereafter. On the basis of transmittance and absorbance characteristics, the six extracts can be grouped into four categories. Junagardh (Gujrat) extract is different from all others and constitutes the first group. Gaya (Bihar) and Arrah (Bihar) extracts constitute the second group. Sonepur (Bihar) extract may constitutes the third group. Hajipur (Bihar) and Patna (Bihar) extract constitute the fourth group. On the basis of absorbance after 900 nm, Sonepur (Bihar) extract may also be characterized to form a distinct group. Graph 1 presents the spectral characteristics of these four groups between 340 nm and 520 nm. This presentation clearly establishes that maximum absorbance in all these cases have been 380 nm. Out of the four, sharp peaks are, however, obtained in the case of Junagardh (Gujrat) and Sonepur (Bihar) extracts.

When colourless extracts not treated with H_2SO_4 were subjected to spectrophotometry, it became clear that λ-max would have been obtained well before 340 nm ultraviolet region (Table 1). However, Junagardh (Gujrat) extract remained distinct from all others showing absorbance between 340 nm and 460 nm. After 460 nm, the turbid extract showed cent per cent transmittance while in case of others absorbance also took place after 460 nm. Of these results also, the extracts may be categorized into three or four groups. Though one may not become confirmed by these primary studies, it is, however, very indicative of the differences in concentration of constituents in different extracts. Further studies are needed to know more about them, Ultra-violet spectroscopy would be helpful in identifying the λ-max point. IR spectrophotometry may be helpful in identifying the constituents of the extracts in terms of their qualitative and quantitative differences (Eglinton, 1970).

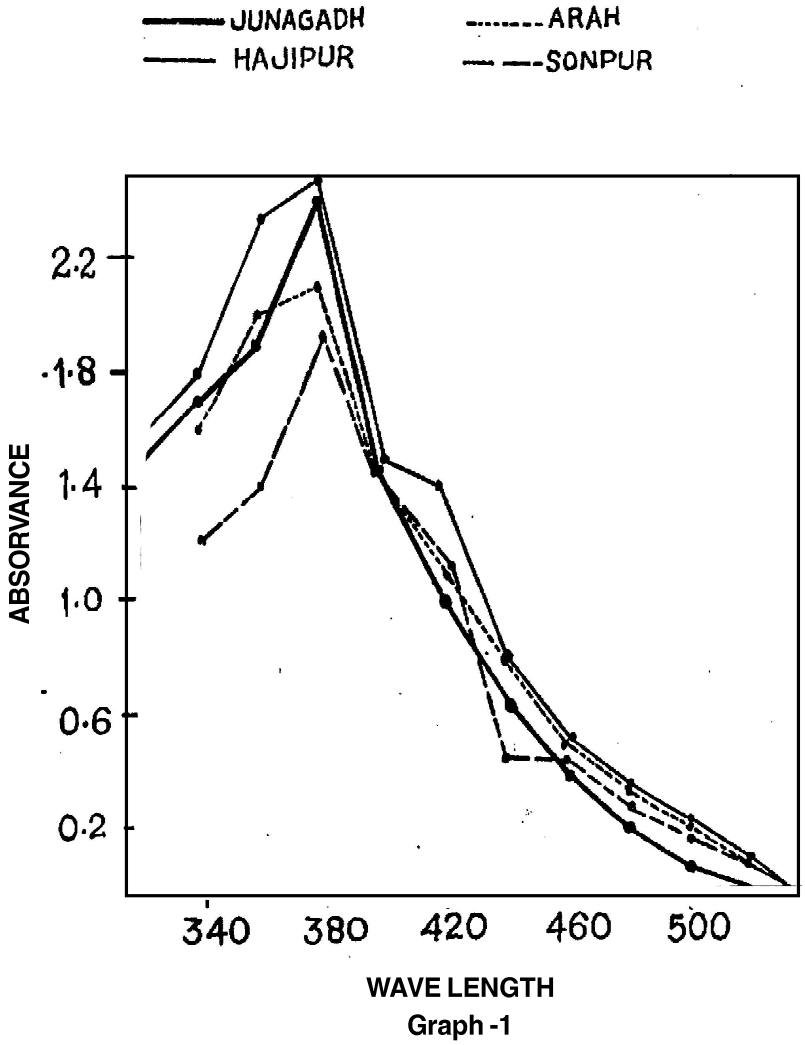
Variations have been found at intraspecific levels in many plants (Kumar and Sinha. 1987). Wakhloo (1963) found variations in alkaloid content of the roots of *Rauwolfia serpentina* growing in different ecological habitats. Chennaveeraih and Rajdan (1980) found differences in the distribution of the phenolic compounds in the *Garcinia xanthochymus*. Many such instances are found in the literature. Doroganyevszkaja (1953) found that the wild content of Coriander Linalool is variable in different climatic and edaphic conditions. It has also been found that a medicinal plant shows an average content in the center of the area of growing, the content becoming variable away from the center (Singh, 1991).

Many triterpenoids are known from the plants and they can be divided into four groups of compounds i.e. triterpenes, steroids, saponins and cardioglycosides. Sterols are triterpenes having cyclopentane perhydrophenanthine ring system. Sitosterol, stigmasterol and campesterol are more or less ubiquitous in higher plants (Harborne, 1973). Saponins are glycosides of both triterpene and sterol and have been detected in over 70 families of plants (Basu and Rastogi, 1967). They are known to have soap like property.

When the extract of *Caesalpinia crista* is shaken in a test tube, soap like foaming is found. It distinctly indicates the presence of saponins. Though TLC and GLC are the tested chromatographic techniques for the separation of triterpinoids whose identities are confirmed by meting point, Rotation, GLC, MS, IR and NMR spectroscopy; paper chromatography has also been found useful, [Basu, 1961; Kawasaki and Miyahara, 1963] for the separation of steroids and saponins.

In the present case of chromatographic studies, acetone-butanol-water in 1:2:10 ratio was found to be most suitable solvent for the run of seed extract on the paper chromatogram. Butanol -water and acetic acid (BAW) showed separation of only one compound with Rf equal to 0.5 and the spot gave carmine colour in sunlight after being treated by the reagent 50% H_2SO_4. Acetorie-butanol-water separated three compounds with Rf... equal to 0.5, 0.67 and 0.82 respectively. These spots respectively gave carmine colour, brown and deep brown or straw orange colour after being sprayed with 50% H_2SO_4.These three spots were consistently found in the cases, i.e., all the extracts of seeds from all sources. No differences were found in the number of spots or their Rf. This is probably because of the fact that the paper chromatogram has not been successful in separating all the constituents. It might also be because of the fact that the seed extract from the different sources differ in quantitative measurements in the constituents and not in the qualitative characteristics. Spectrophotometry study indicated such difference in the content of flavonoid glycosides in different taxa of *Lycopodium*.

From a recent study, it is evident that different types of triterpenes and diterpenes are present in *Caesalpinia crista* (Pascoe *et al.*, 1986). Mixture of several compounds poses problem during separation and careful separation procedures are thus needed to achieve the objective in lull totality. Therefore, combination of spectrophotometry and complicated chromatographic separation would be most useful. However, it is beyond doubt that quantitative differences in phytochemical substances exist which, however, not negate the possibility of qualitative differences. During this study, it was noted that Junagarh (Gujrat) seeds were bigger in size and contained more concentrated and turbid contents which extracted in water giving more foetid smell and more foaming after shaking.



Graph -1

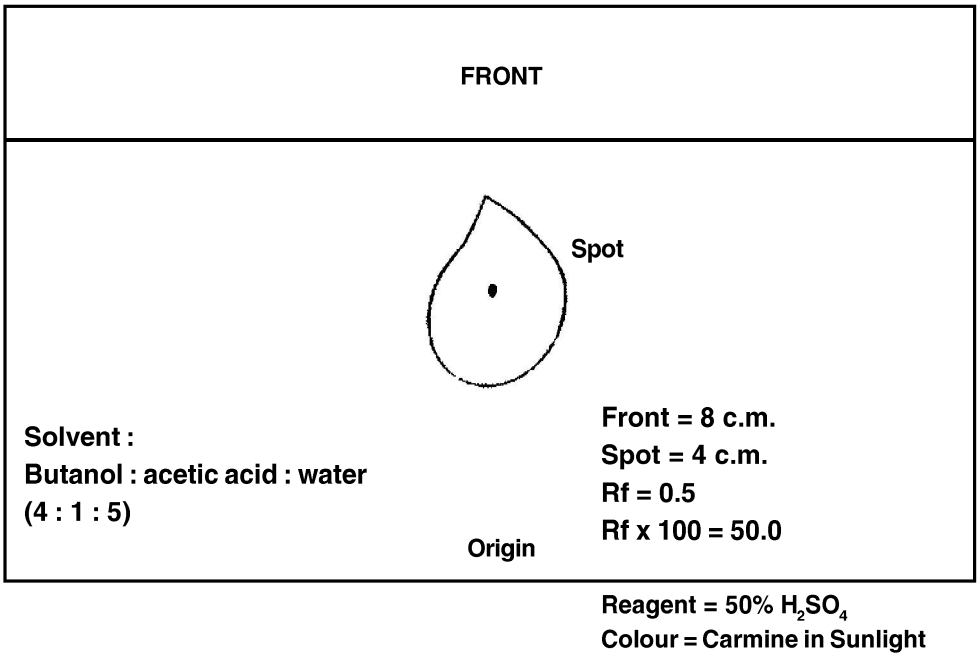


Illustration -1 : Computer Reproduction showing separation of single spot from seed extract on PC

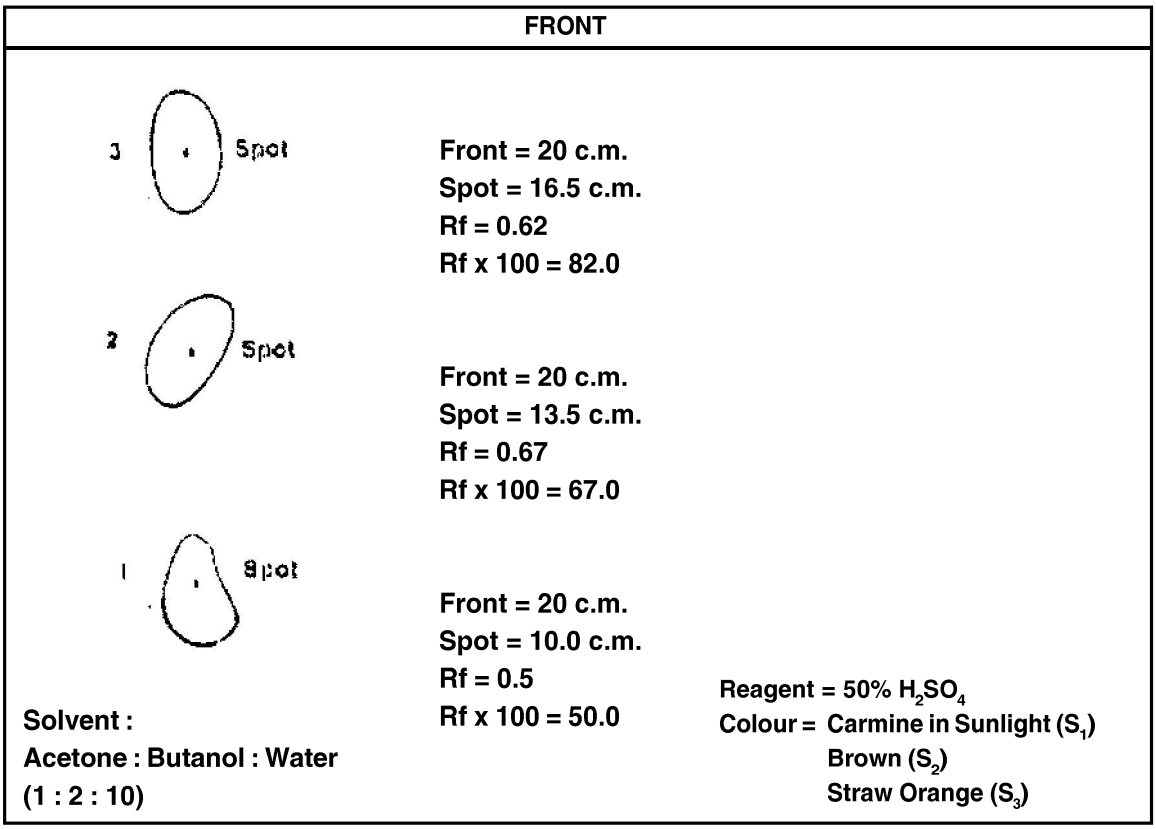


Illustration -2 : Computer Reproduction showing separation of three compounds from seed extract on PC.

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