Indian Journal of Natural Products and Resources
Vol. 8(2), June 2017, pp. 167-171

$K_2Fe(SCN)_6$ from *Crassocephalum crepridioides* (Benth.) Moore. with X-ray florescence spectrometry and X-ray powder diffractometry

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Received 08 February 2016; Revised 09 February 2017

Crassocephalum crepridioides (Benth.) Moore. known as *Terapaibi* in Manipur, India has been investigated for its minerals, trace minerals, and compounds in dried form and in water extract. The elemental analysis was carried out with X-ray florescence spectrometry and the confirmation of compounds isolated was done with X-ray powder diffractometry. The analysis showed the presence of K, Ca, Fe, Cl, S, P, Si, Mg, Na, and $K_2Fe(SCN)_6$. The presence of elements potassium, iron, calcium in *C. crepridioides* correlates to its traditional medicinal use as herbal hemostat, control for hypertension, and immune enhancer in Manipur.

Keywords: *Crassocephalum crepridioides,* Cystic fibrosis, $K_2Fe(SCN)_6$, Trace elements, X-ray powder diffractometry.

IPC code; Int. cl. (2015.01)- A61K 36/00

Introduction

Crassocephalum crepridioides (Benth.) Moore. also known as *Terapaibi* is a herb, which grows in the wild in Manipur, India. People of Manipur use it as a traditional medicine for curing many ailments. The juice of leaves is applied to cuts which control bleeding by helping in blood clotting, leaves are also used for treatment of ulcer, controlling hypertension and boosting the immune system[1,2]. The leaves are taken in raw form with meals. Some medicinal property of the plant is assumed to be due to the trace elements present in the plant, which is highlighted in research conducted on *Ficus pomifera* Wall by the authors[3]. Role of elements like calcium in prevention of bone diseases, K in maintenance of heart, and Fe in prevention of anemia has been reported.[3]

Phytochemical screening of *C. crepridioides* has been studied earlier and antioxidant, antiinflammatory, and cytotoxic activity of the crude extracts have been analyzed[4]. It was observed that the ethanol extract had maximum activity. Another study reported the presence of Na, K, Mg, Ca, Fe, Zn, and Cu using Atomic Absorption Spectrometry (AAS) in the plant[5]. Analysis by AAS is limited as most of the elements in the periodic table are not analyzed due to lack of Hollow cathode lamp for analyzing them[6,7]. X ray florescence (XRF) has been used for elemental analysis in the field of quality control procedures and research concerned with plant samples have advantages for scanning most of the elements in the periodic table[8]. XRF is a non-destructive method used for studying the elemental content of plants and can be used for elements that are difficult to be determined by other analytical methods. Research on using different configuration of XRF spectrometers as an analytical tool to determine chemical composition of vegetation matrices has been reported. Advantages include direct analysis on solid samples, multi element analysis capability, and ability of qualitative and quantitative analysis. The study of minerals and trace element contents of plants that are consumed as vegetable or as an herbal medicine is in demand. Human body requires minerals, which are essential for almost all aspect of body function. Such elements are obtained from our food, deficiency of which results in a variety of symptoms including bone and muscle abnormalities, metabolic disorders, and nervous system disturbances[9-12]. The strong evidence from traditional medicinal uses along with the research report on *C. crepridioides* and medicinal property of elements, lead our research for medicinal trace elements and chemicals in dried leaves and in

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water extract, respectively. The present study reports the elements and a compound $K_2Fe(SCN)_6$ in *C. crepridioides* using its characteristic energy of excitation. To the best of the authors' knowledge, identification of inorganic species $K_2Fe(SCN)_6$ as a possible compound contributing to the immunity and prevention of cystic fibrosis has not been reported so far from *C. crepridioides*.

Materials and Methods

Plant Material

The leaves and young buds of *C. crepridioides* were collected from the North Eastern part of Manipur. The plant was identified by Dr P Kumar Singh Professor of Botany, Department of Life Sciences and a voucher specimen (000207) has been deposited in Manipur University Museum of Plants, Manipur, India.

Sample preparation for XRF analysis

The leaves of the plant collected were air dried in the sunlight and then, the dried leaves were powdered. The powdered sample was sieved using sieve with mesh size 300. The trace elements in the sample were identified using Axios XRF spectrometer PANlytical, Model AXIOS XRF spectrometer, the Netherlands in the powdered form.

Identification of trace elements by XRF

Here in this work, the qualitative aspect of ED-XRF has been used for identifying elements in the plant leaves sample. The reverse bias of 800 V was applied across the semiconductor junction of Si (Li) detector and the resolution of the detector was 150 eV at 5.9 k eV. The sample was powdered finely and mixed with cellulose as a binding material to make self-supporting pellets weighing 300 mg. The sample was placed in front of an XRF spectrometer. For the excitation of the samples, Cd^{109} radioactive source was employed in annular geometry to prevent the direct exposure of the excitation source to the detector and minimize backscatter interference. The Cd^{109} emits Ag K X-rays (energy 22.25 k eV). The X-ray spectra were recorded for a counting time of 2000 seconds and stored in a PC based multi-channel analyzer for further offline analysis [11-14].

Sample preparation for powder XRD analysis

The remaining portion of the powdered leaves was extracted with petroleum ether, chloroform, and methanol at room temperature successively.

Isolation and separation of the white crystals

The extracts were concentrated with vacuum Rotavapor (Buchi) to paste form. The methanol extract showed large amount of whitish compound. The compound was soluble in water. A white crystal was isolated from the methanol extract by extraction of the white compound with water by solvent extraction technique from the methanol extract paste[15]. The water extract was concentrated and filtered repeatedly, purified till white compound is obtained. The white compound was purified by recrystallisation.

Qualitative analysis of white crystal by powder XRD

X-ray powder diffractometry is unique as it combines absolute specificity with a high degree of accuracy. It is a powerful technique for identification of crystalline solid phases by unique diffraction pattern. Powder XRD has been used for phase identification and quantitative phase analysis. Technique such as application of whole powder-pattern decomposition that uses single line intensity is implemented. Another application is based on whole powder-pattern fitting and other techniques used for qualitative phase analysis by using Reitveld refinement. The white compound was purified by recrystallisation for recording powder XRD pattern. The white crystals were finely powdered and loaded on the system with a sample holder. The spectra was recorded from 5 to 90, 2 theta. Diffractometer system was XPERT-PRO from PAN analytical. The spectrometric conditions at which the analysis was carried out were - step size [°2Th.] of 0.0500', Anode Material was Cu, K-Alpha1 [Å]:1.54060, Generator Settings were at 30 mA, 40 kV with scan step time [s] was 2.0000, divergence slit size was 0.9570, and receiving slit size [mm]: was 0.2000. The Bragg-Brentano focusing geometry was used[16-18].

Results and Discussion

The minerals present in the leaves of *C. crepridioides* were calcium, potassium, sulphur, phosphorus, silicon, chlorine, and sodium. Iron and magnesium were the trace elements identified. The trace elements and minerals were determined qualitatively with the help of X-ray florescence spectrometry. The peaks of the elements in the XRF pattern are shown in Fig. 1. Since XRF is a very sensitive technique which can detect all elements except for some elements like hydrogen, it can be concluded that other elements in the periodic table are absent in *C. crepridioides*.

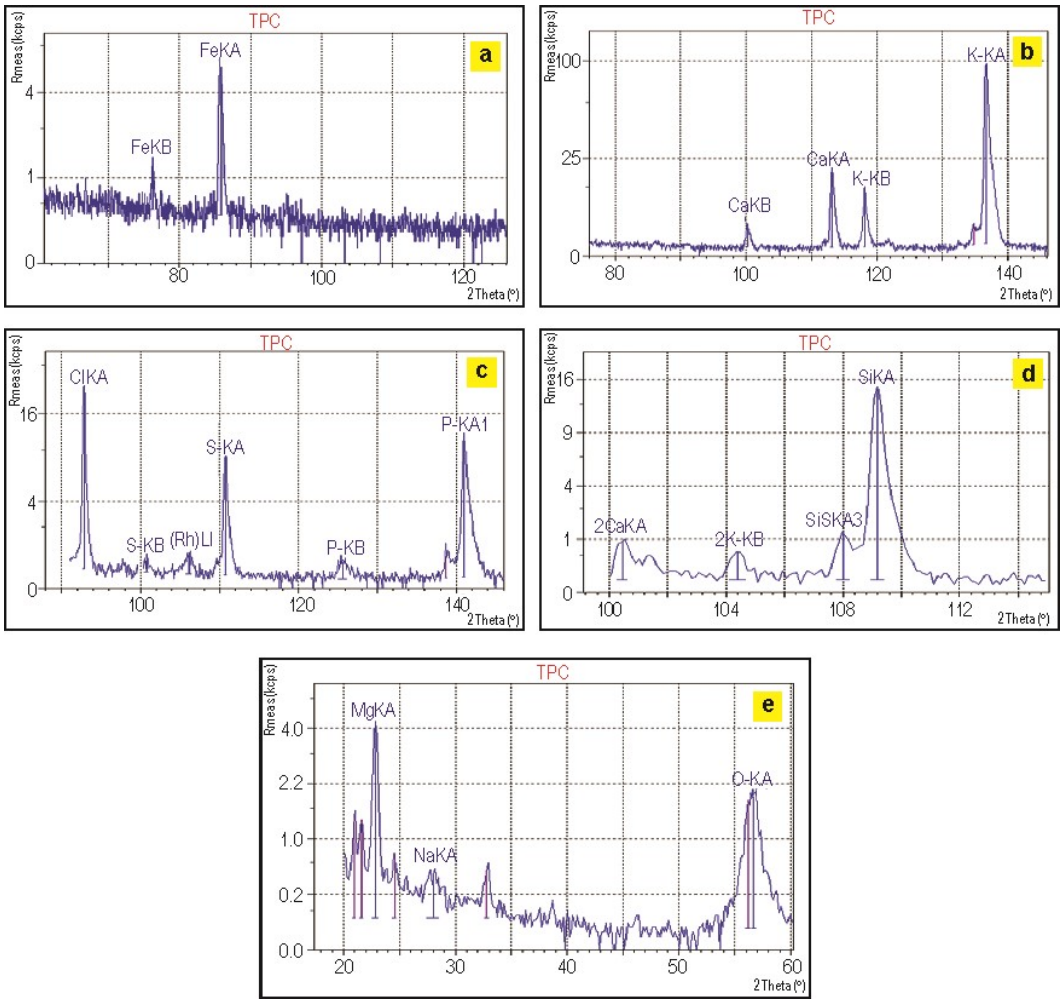

Fig. 1 — XRF pattern of Iron ; calcium and potassium; chlorine, sulphur and phosphorous ; calcium, potassium and silicon; and magnesium and sodium of *C. crepridioides* species from India

In a previous study on *C. crepridioides* growing in Indonesia[5], Zinc was also detected in the leaves of the plant with AAS technique but zinc is not detected in the present study. Further, Cl, P, Si have been reported that were not reported in the above research. This also further illustrates the difference in elemental composition with different geological and environment factors. The leaves had K in appreciable amount, which manifest its traditional use in controlling hypertension. Appreciable amount of K decrease the risk of heart diseases and high blood pressure. Ca helps in maintaining bone health and contributes to blood clotting supporting its use as herbal hemostat. As Ca is required for conversion of prothrombin to thrombin which makes the blood clot and cuts are healed.

The powder XRD pattern of the white compound isolated from methanol extract was recorded. The powder XRD pattern was compared with the patterns of International Centre of Diffraction Data (ICDD) with software HIGHSCORE and matching pattern was found with $K_2Fe(SCN)_6$ with reference code 00-036-0786 with PDF index name potassium iron thiocyanate. Both the patterns are illustrated in Fig. 2. This method is highly accurate compared to the traditional wet test methods that require larger sample amounts. Iron and magnesium are also present which prevents anemia.

In powder XRD, the peaks of the sample and standard reference matched each other perfectly. The peak at 2θ 32 and 33 are not seen in pattern sample, though present because of high sample intensity. The peak at 2θ 45 is assumed to be from impurity.

Thiocyanate is known to be an important part in the biosynthesis of hypothiocyanite[19]. Hypothiocyanite is biosynthesized by lactoperoxidase[20]. Hypothiocyanate

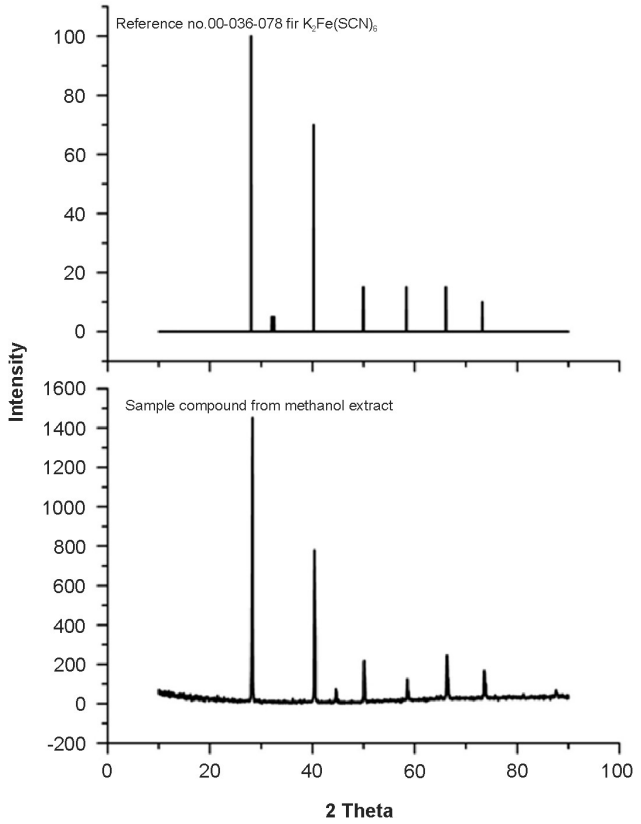

Fig. 2 — Powder XRD pattern stacking compound with reference potassium iron thiocyanate

has bactericidal potential and contribute to the immunity of the host. A sustained supply of thiocyanate (SCN^-) that turns into Hypothiocyanate ($OSCN^-$) from the blood stream to airway mucosal surface prevents cystic fibrosis. The SCN pool is believed to arise from two sources, from detoxification of cyanide and more significantly from the diet[21]. Glucosinolates of plant tissues are rich sources of SCN and thus, intake of such plants prevent cystic fibrosis[22]. Hence, this compound may be acting as source of SCN, which relates to its traditional use in many ailments like cold and healing of wound.

The presence of the $K_2Fe(SCN)_6$ in the methanol extract might be correlated to the cyotoxicity reported in the ethanol extract of leaves of *C crepridioides* of South Africa[4].

Conclusion

Thus, from the results, it can be concluded that the high content of potassium may justify the folklore use of leaves of *C. crepridioides* for control of hypertension. The appreciable amount of calcium explains its use as herbal haemostat. Iron and magnesium is also present that may prevent anemia. The difference in elemental composition of *C. crepridioides* species from Indonesia and India may be attributed to the different geographical conditions in the two places. This plant might be used as source of SCN, which might be the reason behind the traditional use of this plant leaves for boosting the organism's immune. The high content of compound $K_2Fe(SCN)_6$ is believed to contribute to the medicinal properties. Further studies on the complex and its activity are needed.

Acknowledgement

The authors acknowledge the Physics Department of Manipur University for the Powder XRD facility, SAIF, Guwahati for XRF facility in this research work, Prof. P Kumar Singh for the authentication of plant from the herbarium collection in Manipur University Museum of Plants, Manipur, India.

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