



Identification and Comparison of *Xanthium Strumarium L.* as Crime Scene Evidence in Forensic Rape Case by Maceration and ED-XRF Techniques

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Abstract

Forensic botany is utilization of plant materials in crime investigation and court. It utilizes traces of plant material as physical aid for criminal investigations. The physical aids found at the crime scene can throw light in determining circumstances happened at the crime scene. The physical clues left therefore decides whether victim was (and was not) involved at the crime scene. These types of clues are challenges for forensic experts to collect evidences and make analysis from crime scenarios. As rape is a contact crime. Herein we report a forensic rape case in which the weed material of *Xanthium Strumarium L.* adhere on accused cloths and victim cloths and the weed material of *Xanthium Strumarium* collected from crime scenario all of them belongs to same plants materials. First we performed the physical fit of half of the weed piece observed on accused cloths and another half part of weed observed on victim cloths matched exactly with each other. A small quantity of macerated tissues may be mounted in glycerin and observed under microscope. The ED-XRF spectrometry is suitable analytical tool to detect the trace elements presents in all the samples of weed of *Xanthium Strumarium L.* A single weed material of *Xanthium Strumarium L.* provided link between the criminal, the victim and the scene of occurrence.

Keywords: *Xanthium Strumarium L.*, Rape, Weed, Physical fit, Maceration, ED- XRF.

Introduction

The word "forensic" is derived from the Latin term "forensis" which means "of or before the forum." Forensic science involves application of science to criminal and civil laws. Factual scientific evidences found at crime scene could be proved as legal evidences¹. Botany is the branch of science which studies the plants; it consists of many branches such as taxonomy, morphology, anatomy and ecology and DNA analysis which are important in the forensic science applications². Forensic botany is utilization of plant materials in crime investigation and court. It utilizes traces of plant material as physical aid for criminal investigations provides possible links between a suspect, victim and a crime scene³⁻⁶. *Xanthium Strumarium L.* is common annual herb weed plant with two strong hooked beaks found in India⁷⁻⁹. *Xanthium Strumarium L.* grows throughout the year on waste lands as weed plant. In Maharashtra locally it is called as 'Gokharu', in Unani as 'Kutta Jhad' and in Ayurveda it is called 'Shankeshwarab' or 'Arishta'. Genus *Xanthium* includes 25 species of American origin and two species namely *X. Indicum* and *X. Strumarium* found in India and considered as worst weed. The seed are easily spread because of their light weight, ability to float, 'hitchhike' on humans and animals. *X. Strumarium L.* plant is rich source of mineral K (potassium), flavonoids, catechol, alkaloids,

cardenoloids and amino acids. The plant roots and fruit is medicinally important in Ayurveda remedies¹⁰. The identification of wood and plant material in terms of commercial, forensic, archaeological and paleontological views is important. Identification is difficult because of the high diversity and similarity of many genera. The information about genetic origin, the formation time and environmental conditions could be found in wood and bark present in stems, branches, roots of trees, shrubs and herbs¹¹. Scientific evidences play important role in today's judiciary system. Many high profile criminal investigation conclusions almost entirely based on scientific evidences. Edmond Locard (1877-1966)¹² is known as the "Sherlock Holmes of France." He was lawyer and a physician. He had established first "Police Laboratory" in 1910 with the Police Department of Lyon, France¹². Edmond Locard stated that "Every contact leaves a trace" which is known as "Locard's Exchange Principle", the basic principle of forensic science¹³. If any two objects come into contact, exchange of material will take place between them even if it is only a few atoms or molecules. Every criminal carries trace evidence with him/her from the scene of crime by which he/she can be linked with the crime. Every incident that is a crime, accident, natural disaster or other leaves traces or clues at the scene. This is the basic principle of trace evidence collection at a crime scene. Forensic science itself begins at the crime scene¹⁴. Anything

recovered from crime spot or at related locations could be generated physical evidence as part of a crime (Indian Evidence Act-1872)¹⁵. The possible links between a suspect, victim and a crime scene and whether victim was (and was not) involved could be established on the basis of the factual clues left at the crime. The biological evidences could be successfully utilized in investigation of rape cases. It is important to provide shielding to internal and external interference with the proper and successful investigation of rape cases. According to Section 376 of IPC, Rape is a contact crime in which the unlawful sexual intercourse with woman is made by man without her consent¹⁶. Rape is one of the most frequently committed violent acts. It is estimated that more than 60% of rape cases are unreported. Reported rape cases has lower conviction rate than for other violent crimes. The protection of children from sexual assault offences, sexual harassment and pornography at every stage of the judicial process could be provided by The POCSO Act-2012. The child-friendly mechanisms could be employed for reporting, recording of evidence, investigation and speedy trial of offences through designated Special Courts¹⁷. The unnatural sexual offence like anal intercourse between men or between man and woman is called Sodomy or buggery¹⁸.

Age of Victim (Woman): No age is safe for Rape. In India a child of 5 year or less and old woman of 70 year or more have been raped.

Age of Accused: In India there is no age limit. For the child, Court decides his potency and knowledge regarding crime, good non violence and mental status through medical examination. Even old men commit rape on young or minor girls.

Punishment of Rape: Under section 376 IPC: Imprisonment of not less than 7 year or life or 10 year with or without fine.

Materials and methods

This study was carried out in the Regional Forensic Science Laboratory, Nashik. In most of the sexual offence cases received to forensic science laboratory, it was seen that an accused persons raped on victim due to personal contact between them. They left some physical clues at crime scene and examination of biological evidences left behind on the body of accused person, victim or on his/ her garments and at the crime scenario, an accused could be easily pointed out. Many of the cases were received under the IPC section 376 (F) (I), 377 and POCSO Act-2012. Here in such case a five year minor victim has been raped by her closely related neighbour at his farm. The medical officer sent the medical samples of victim and accused while the investigating authority sent the garments of victim and accused at the time of incident for chemical analysis and, they also sent the weed (seed with throne on outer side) material found on the crime scenario and asked an interesting question like a fragments of weeds adhere on victim and accused cloths if observed then all of them are match with each other's and belongs to same plant material or not? and it is the most challenging task for the forensic expert.

Herein such analysis first we observed the weeds and their fragments on victim as well as accused cloths i.e. the botanical findings. Moreover the blood stains and semen stains were detected on victims and accused cloths i.e. the biological findings. ABO blood grouping was performed by absorption-elution method. Blood stains detected on accused cloths and victims cloths were matched with victim's blood group where as semen stains detected on victims cloths and accused cloths were matched with accuser's blood group.

Herein present study first we performed the physical fit and study the morphological and microscopic examination and a comparison by Maceration and Elemental Analysis of fragments of weed material observed on accuser's cloths, victim's cloths and weeds collected from crime scene spot by Physically, Botanically and Chemically.

Authentication of plant material: *X. Strumarium L.* weed material and their fragments found on cloths of accused person and at crime scene were brought to laboratory and botanically identified and authenticated plant species as *Xanthium Strumarium L.* by Mali with the help of standard flora¹⁹.

The forensic evaluation of the fracture marks involved the examination of the unique features that are present on two pieces of material once they have been separated. The individualizing characteristics on the fracture surfaces have been evaluated in materials involved at the crime scene proved that the two parts were once a whole provided the connection between crime and suspect. The fracture features exhibits enough evidence to prove that the two halves were once a whole²⁰.

Macerated plant materials are used for the morphological and microscopic examination of exhibits by plant anatomy. A small quantity of macerated tissues usually mounted in glycerine and observed under microscope²¹. "Small-scale pulping" or "test-tube pulping" is referred as the maceration process. Mixture of formaldehyde and nitric acid was employed for maceration of fibres²². Han *et al*²³ developed and compared macerated fibres with fibre length measurement techniques.

Maceration Process: The fragments of *X. Strumarium L.* weed material observed on accused or victim's cloths, collected from crime scenario were collected and cut into small pieces of 1 mm thick size by cutter knife. These tissues were dipped into freshly prepared macerating fluid in separate test tubes. Jeffrey EC utilized freshly prepared mixture 8 to 10% nitric acid and chromic acid for maceration²⁴. The test tubes were slightly heated and wood samples gets macerated due to chemical reaction on wood sample material. The fluid mixture is prepared by mixing 10% chromic acid and 10% nitric acid. The tissues were left in the macerating fluid for about five days. Then plant tissues were teased with dissecting needles. If the cells do not separate readily, then the tissues were left in the macerating fluid for another day. If the cell separate easily, they are readily

taken for next step. The macerating fluid was filtered off and washed with water to remove the acids from the macerated material. The macerated material was ready for temporary mounting. Canada balsam using a cover glass was used for mounting. For slide preparation, 20% safranin was used fibre staining, washed with distilled water for de-staining of excess safranin. For slide observation, some amount of glycerol was placed on a standard glass slide and allowed for air drying.

ED-XRF: A tool for forensic analysis: The root and fruit of *Xanthium Strumarium L* is used as medicine. XD-XRF is the microscopic technique used for qualitative evaluation and confirmation the structural details of drugs from the *Xanthium Strumarium L*. It could be used to study the constituents by application of chemical methods. Virtually anything can be presented for analysis in the course of forensic investigation. ED-XRF spectrometry is the best instrumental techniques utilized for any state sample, liquid, solid, thick, intermediate or thin with sufficient sensitivity. The ED-XRF could be used analysis of aerosols, waters, sediments, soils, solid waste, medical, vegetables, plants, other environmental samples, determination of trace elements of different samples such as some lichens, tobaccos, coals, sea sediments, red mud, medicinal plants, blood, ash of power plants, etc.²⁵⁻²⁸.

Experimental: Microscope: Observations were recorded using Motic Digital Binocular Microscope (Model: BA210, 10 X magnifications with Motic Image plus 2.0 ML software). Macerated fibres in 10 % nitric acid showed separation of fibres without splitting.

ED-XRF: Energy Dispersive X-ray Fluorescence Spectrometer (Make: OLYMPUS- INNOVX X-50 software with version: 2.5)

Chemicals and Reagents: All the chemicals (chromic acid, Nitric Acid, Glycerol) used for this study purpose were AR (Analytical Reagent) grade and purchased from Sigma Aldrich.

Results and discussion

Morphological and Anatomical features of Fruits of *Xanthium Strumarium L*: Latin name: *Xanthium Strumarium Linn.*, Synonyms: *Xanthium Canadense*, Family: Compositae, Kingdom: Plantae-Plant, Habitat of herb: Cultivated crops, waste land and pastures, river banks, lake shores, etc., Fruits of *Xanthium Strumarium Linn.*, Shape: Oval shape covered with numerous hooks., Size: 1.5-2 cm length, 1 cm width, Colour: Fresh fruit green, dried fruit brownish., Herb: Cocklebur,

Known hazards of *Xanthium Strumarium L*: Most members of this genus are poisonous, toxic to grazing animals (Figure- 1-3).

In present study, we utilized the mixture of 10% chromic acid and 10% of nitric acid solution for maceration. The weed samples were treated with 10% nitric acid and 10% chromic acid that dissolved the middle lamella and separated the

cells/fibres from one another. Anatomical features include the arrangement, distribution, frequency and size of the various cell elements viz., vessel, axial parenchyma, ray parenchyma and fibres in the weed. These characteristics included both macroscopic and microscopic characters. We identified that the weed material observed on accuser's cloths, victim's cloths and weed collected for crime scenario found same after the careful observation of weed material, the physical fit. We made the morphological and microscopic examination, the elemental analysis and a comparison of weed material, physical fit and weed material collected at crime scene.



Figure-1: Fresh fruit.



Figure-2: Dried fruit.



Figure-3: Control sample from crime scene spot.

Physical Fit: (Morphological study): Weed materials adhered on victims; accused cloths and crime scenario were shown in Figure- 4-5.



Figure-4: Victim cloth (Frock).



Figure-5: Accused cloth (Full Shirt).

Half of the part of weed material observed on victim cloth and another half part of the weed observed on accused cloths, both the halves were matched perfectly with each other and constituted a single weed material.



Figure-6: Measurement of both the weed material.

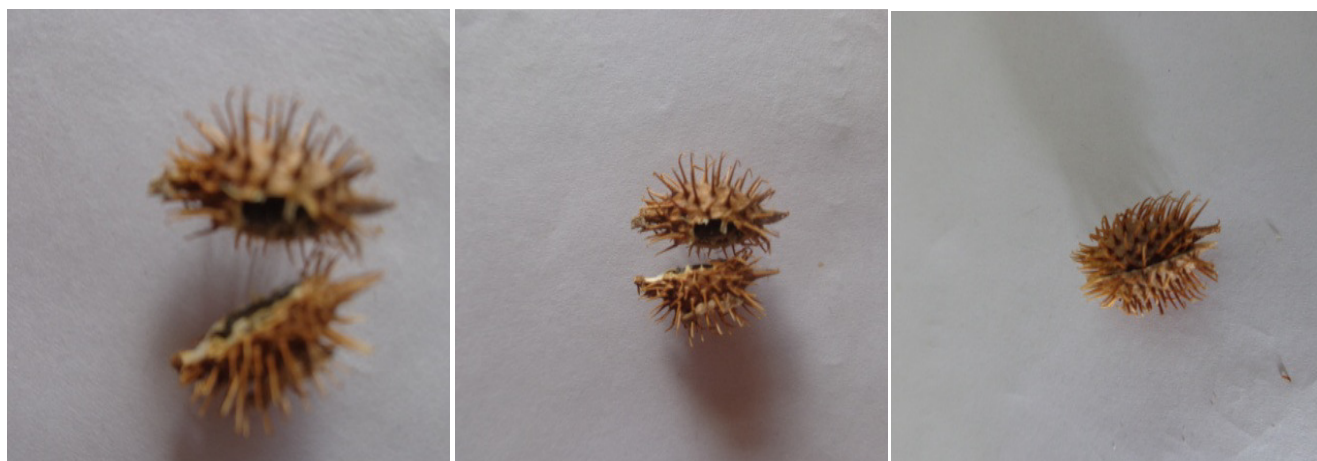


Figure-7: A perfect physical match of both the weed material.

Both cut side has characteristic irregularities and both pieces were matched with each other as shown in photograph (Figure-6, 7). Figure-7 shows both pieces matching with each other mechanically and reconstructed in one article that is a single weed.

Maceration of weed materials: Microscopic Identification of Wood: The microscopic identification of plant species is an established method routinely used to identify weed type. It consists of macroscopic and microscopic examination during with the genus and in many cases the species of weed samples can be determined exactly based on their anatomical structure.

Nitric acid is cheap, easy to handle and fast resolving agent utilized to break down the middle lamella for separating the cells. The morphological and microscopic result revealed that anatomical arrangement, distribution, frequency and size of the various cell elements found same for test samples (Figure-8, 10).

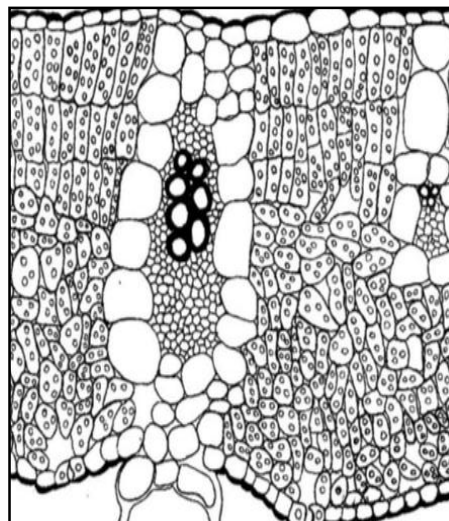


Figure-10: Lamina.

Xanthium strumarium L. exhibited distinguishing characteristic features by which it could be easily distinguished from other plants. The important plant characteristics included scabrid surface, unisexual capitula, root with pith, anomalous secondary growth of the stem, primary vascular bundles in palmate fashion and presence of spiny trichomes and scales.

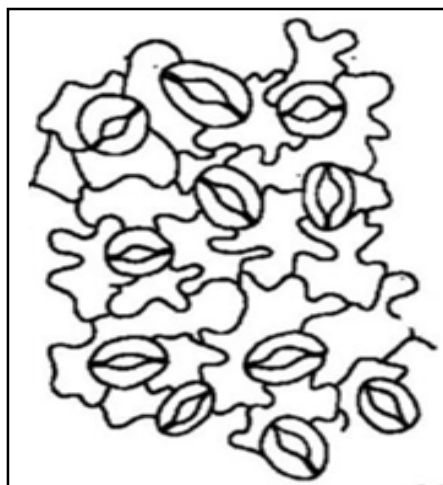


Figure-8: Upper Epidermis.

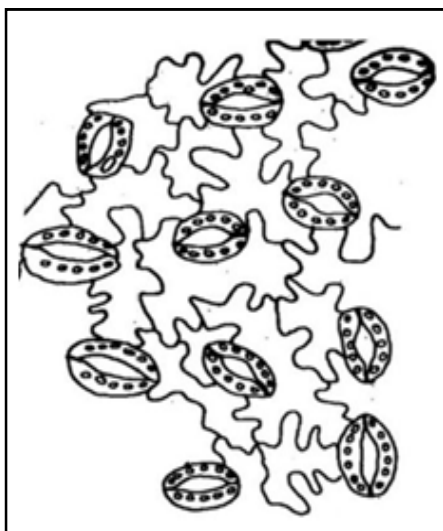


Figure-9: Lower Epidermis.

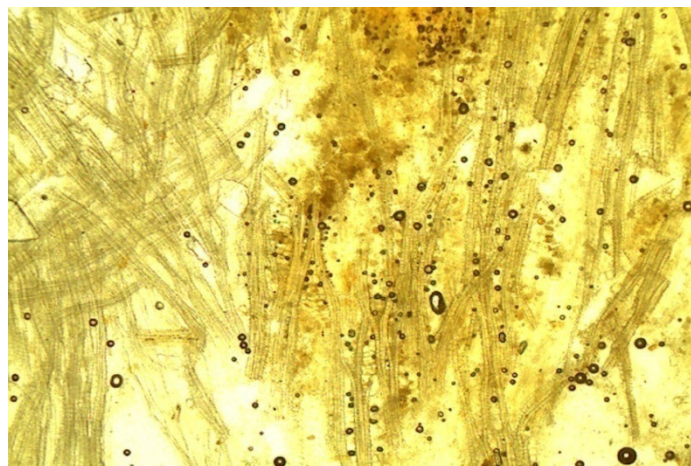


Figure-11: Macerated weed material from victim's Cloths.

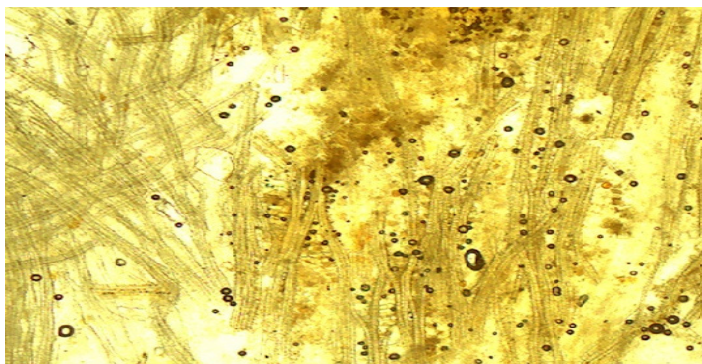


Figure-12: Macerated weed material from accuser's Cloths.

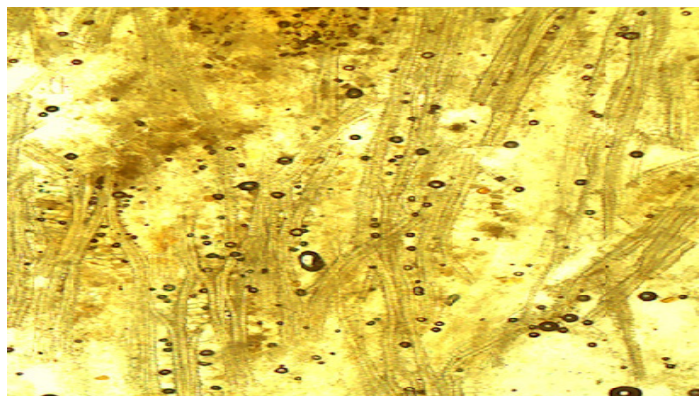


Figure-13: Macerated weed material from crime scene spot.

ED-XRF: ED-XRF involves irradiation of the sample with a beam of X-rays that induces fluorescence in the atoms present in the sample material. The X-rays are then re-emitted as a lower energy X-rays. The X-rays of different and unique energies was emitted by every element. The intensity of X-rays found proportional to the concentration of particular element in the sample.

The energies emitted could be detected and analysed by detector system. Intensities of X-rays emitted were measured and the concentration of the different elements in the sample was determined.

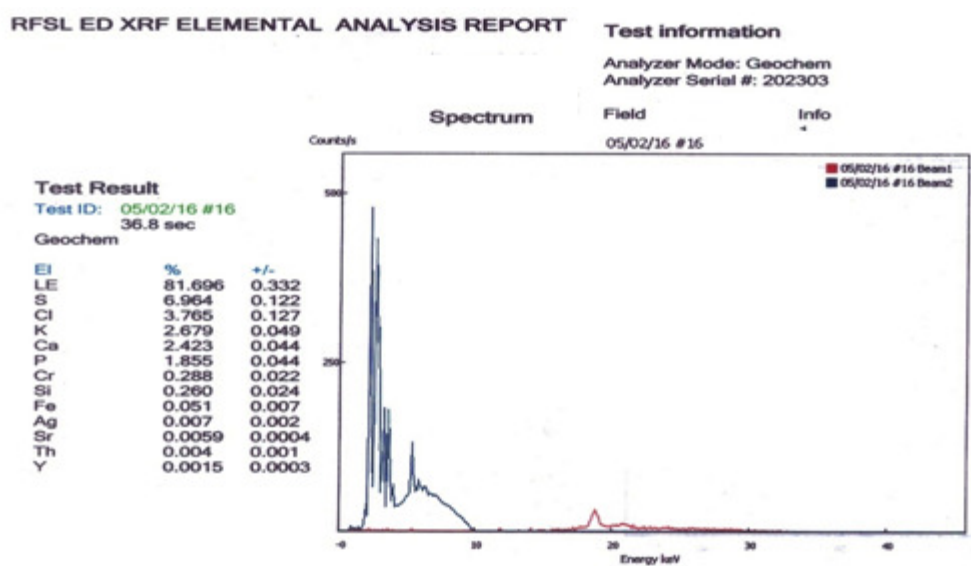


Figure-14: ED-XRF spectrum of weed material from victim's Cloths.

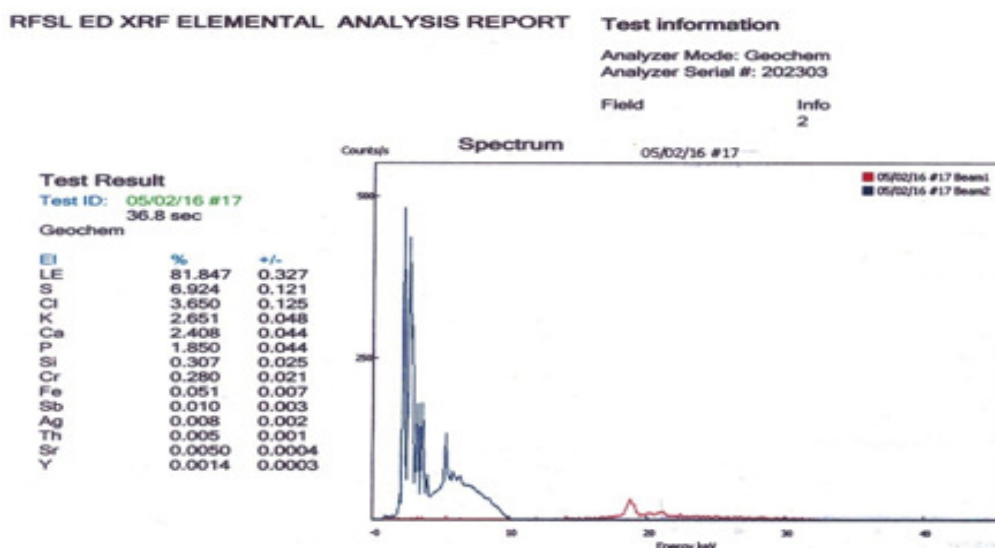


Figure-15: ED-XRF spectrum of weed material from Accuser's Cloths.

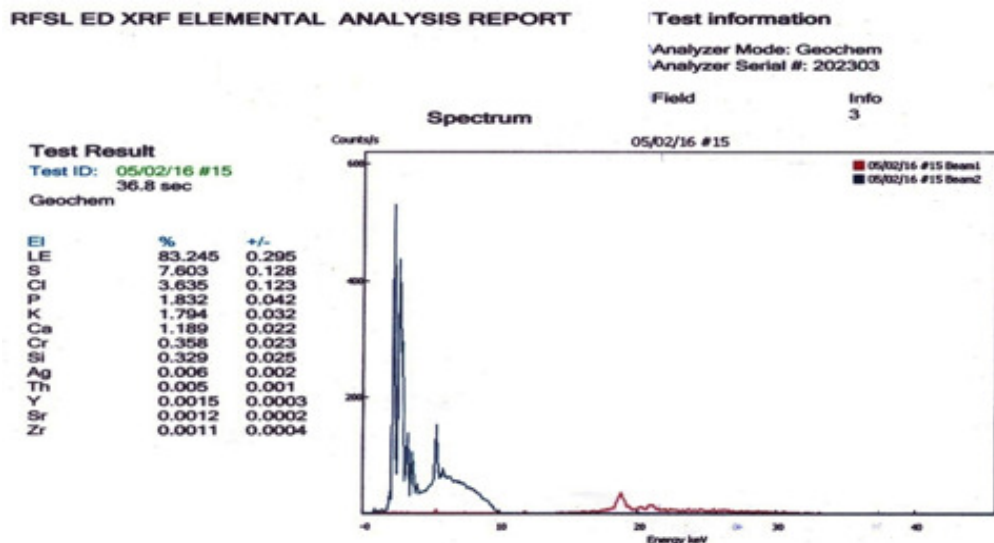


Figure-16: ED-XRF spectrum of weed material from crime scene spot.

From Figure-13, 15, it is seen that the overall agreement of the calculated spectra with the measurements is excellent. The plot is linear and the main peaks represent elemental characteristic lines. The advantage of this work is the prediction of absolute count rates found significant and net intensity errors due to peak tailing are small. A adjustment of few percent in geometric factor would improve the agreement at the peaks. All test samples showed same concentrations of trace elements (Figure-14-16).

Conclusion

Our study showed exact situation at the rape crime scene. As the rape is a contact crime, the weed material of *Xanthium Strumarium L.* adhered on accuser’s cloths, victim’s cloths and weed material collected from crime scene spot found was from same plant material. It was characterized by physical (physical fit), botanical (maceration) and analytical (ED-XRF) methods. The weed material of *Xanthium Strumarium L.* are adhered on accuser’s cloths and victim’s cloths directly proved the crime scene spot and hence the crime also. Maceration of wood fibres in 10% chromic acid and 10% nitric acid mixture had advantage of short time, easy to handle and economically beneficial than other methods. The Physical fit of half of the weed piece observed on accused cloths and another half part of weed observed on victim cloths matched exactly with each other. The ED-XRF spectrometry is suitable analytical tool to detect the trace elements presents in all the samples of weed of *Xanthium Strumarium L.* A single weed material of *Xanthium Strumarium L.* provided link between the criminal, the victim and the scene of occurrence.

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