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Assessment of Minerals Component and Characterization of Solanum Dubium Collected from White Nile (Kenana area) in Central Sudan

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Abstract:
Certain members of the Solanaceae family, like the well-known wild plant Solanum dubium "Gubbain," grow abundantly during the rainy season in several parts of Sudan. In the countryside, solanum dubium is used to coagulate milk. It is a thickly developed, bushy pubescent herb that coexists with several species, including S. innacum, Along with S. melongena, S. macrocarpon, and S. esculentum, in northern, central, and western Sudan. locating pure versions of the crude enzyme.
Solanum from the seeds and producing the enzyme commercially for use in cheese manufacturing were the primary goals of research on S. dubium. In addition to other metabolites like flavonoids, solanaceous plants are highly alkaloidal, and this is what gives them their antibacterial activity in all plant parts, including the seeds.
The purpose of this study was to identify the characteristics and chemical makeup of the seeds of the Solanum dubium plant. For millennia, the plant Both raw and refined forms of Solanum dubium have been employed in the production of cheese as a milk coagulant. Due to rennet's restricted supply and expensive cost, dietary restrictions, religious beliefs, or the prohibition of recombinant calf rennet in some nations, these coagulants serve as an alternative to rennet. Plant proteases can be obtained naturally or by in vitro culture since almost all plant tissues contain these enzymes. This makes plant proteases available all the time. The majority of plant coagulants are too proteolytic and generate lower cheeses, which limits their application in cheesemaking.
Several kinds of buffers were used to mix and extract the seeds of Solanum dubium. Throughout the investigation, it was discovered indicated the most consistent, quick, and efficient buffer was 5% NaCl in acetate buffer (pH 5.0). Ammonium sulfate was used twice to filter and fractionate the extract. It was discovered that the S. dubium enzyme has greater clotting and proteolytic activity than other plant enzymes.
The concentration of the enzyme and substrate raised the enzyme's activity continuously. It was demonstrated that the enzyme remained remarkably stable over a broad pH and temperature range (20–90 °C).
Studies on its substrate selectivity revealed that the partially purified enzyme favored amino acid residues at the P1 site that were both hydrophilic and hydrophobic. An aliphatic amino acid (Leu) at the P1 position at the same site enhanced the refined enzyme's catalytic efficiency over an aromatic residue (Phe). Using a Philips Lancashire XL-30 scanning electron microscope at various magnifications, the morphology of solanum seeds was examined. The findings are shown in Figures 5, 6, and 7. Furthermore, the fractionation of structure as shown by infrared spectroscopy is depicted in Figure (8).
Several metals in solanum seeds in this investigation comprised fundamental elements like Ca, Mg, K, and P as well as elements like Mn, Cr, Cd, Zn, and Fe. The sample showed that Mn, Cr, Fe, Cd, Zn, and P flowed along with K, and larger quantities of one and other metals., respectively, were the maximum values permitted. Seed of Solanum samples were gathered from the Kenana region in the White Nile. The levels of metals in solanum seeds are displayed in Tables 2 and 3. The results of the investigation demonstrated a strong positive link between the levels of heavy metals and other metals in Solanum. The findings demonstrated that the kind of solanum coagulant used in the cheese business had no discernible effect on any of the chemical components under investigation.
Keywords*: plant Solanum dubium, (Gubbien) seed, chemical component, solvent extraction, and dubumin enzyme.*

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I. Introduction

Among the many and varied flowering plants in the genus, Solanum are two highly valuable food crops. It includes several plants grown for their attractive blooms and fruit, as well as horse nettles and nightshades.

Numerous growth habits are exhibited by Solanum species, including vines, shrubs, subshrubs, tiny trees, annuals, and perennials. Many once distinct genera, such as Cyphomandra and Lycopersicon (the tomato

family), are now sections or subgenera of Solanum. As a result, the genus currently has between 1,500 and 2,000 species (20, 17, 28).

Figure 1. The entire Solanum dubium plant (left, with green and yellow fruits and dark clusters of seeds).

Scientific categorization	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Asteroids
Order:	Solanales
Family:	Solanaceae
Genus:	*Solanum*
Species:	***S. dubium***

Binomial name ***Solanum dubium***

Fig. 3. Solanum dubium fruits with coat and seed

With more than 2000 species spread over the globe, the genus Solanum is the largest of all flowering plant genera and the largest member of the Solanaceae family (19). The Solanaceae family comprises medicinal herbs (6) that are used to treat a variety of illnesses, including high blood pressure, diabetes, cholera, bronchitis, and laxatives. These plants also contain special alkaloids and other biochemical elements (8).

Plant and its applications

The woody herb Solanum dubium fresen is native to Sudan and is widely grown in its native regions. Ripe fruits are yellow in color, Unripe fruits have a green color and usually dry out on the stem.. Animals avoid eating dark brown seed because of its bitter flavor. In certain regions of Sudan, dairy producers utilize the berries of Solanum dubium to produce Gibna Bayda, a soft white cheese, using milk from goats and sheep (29, 28).

The end product is a crumbly, delicate cheese with a hint of bitterness that is ascribed to the non-specific proteolytic activity of enzymes generated from Solanum dubium berries or the presence of particular alkaloids (3). No studies on the use of plant enzymes to make Mudaffara cheese have been conducted in Sudan (1, 2, 4).

Vegetable rennet becomes extremely important when animal rennet is unavailable, It's not feasible to kill calves for chymosin, or the cheese is meant solely for vegetarians. Vegetable rennet could enhance the nutritional value of cheese made from it, even while the use of animal rennet is prohibited (2,6,8,15,19). Several plants, notably Solanum dubium, are sources of enzymes that coagulate milk. One well-known species in the Solanaceae family is Solanum dubium (Suleiman et al. 1988). It is also referred to as a bushy, pubescent herb plant that grows throughout Sudan. It was discovered that the S. dubium fruit contained a significant concentration of the rennin-like substance (9) that is utilized in the manufacturing of white cheese. Several studies have

demonstrated the benefits of employing In the process of making white cheese, Solanum torvum, and Solanum dubium are used (3,5,4,20).

Cheese made using rennin derived from S. dubium has a lighter, softer, and more compact texture than cheese made with conventional rennin sources, according to Dawla's (2001) research. Recently, there has been a lot of interest in the discovery of a milk-clotting enzyme derived from natural plants that might successfully take the place of rennet sold for cheesemaking. (14). Generally speaking, callus—a disorganized collection of cells—may be produced using extracted plant material. (explants), allowing for the continuation of callus growth during explant culturing and the easy regeneration of entire plants (plantlets) (3,5,4,20)

Their investigation revealed promising outcomes when employing this extract to produce soft white Sudanese cheese. The objective of this study is to examine the chemical makeup and characteristics of seeds of Solanum dubium (5, 9, 16, 27).

Solanum dubium antioxidants:

Due to their strong pharmacological effects and commercial feasibility, plants have been studied for their therapeutic qualities globally in light of current scientific advancements. Many aromatic and therapeutic plants include chemicals with antioxidant properties. Plant phenolic compounds, which can be found in many sections of plants, including fruits, vegetables, nuts, seeds, leaves, roots, and bark, make up the majority of natural antioxidants (Pratt, 1990). Numerous antioxidant substances have varying degrees of anti-inflammatory, ant-atherosclerotic, anticancer, anticarcinogenic, antibacterial, or antiviral properties Sala et al., 2002). The food industry is becoming more interested in crude extracts of fruits, herbs, vegetables, grains, and other plant materials rich in phenolic because they block lipids from deteriorating oxidatively, enhancing food quality and nutritional value (Kahkonen et al, 1999; Rice et al, 1995).

Useful solanum dubium Before employing honey in the dairy and pharmaceutical industries, use it to increase activity. The earliest medical texts and religious testaments both attest to honey's healing properties. Honey is currently demonstrating potential at the research level to reduce skin cellular damage after radiation treatments (21, 9, 16).

II. Materials And Methods

Preparing samples of Solanum dubium

Between December 2016 and January 2017, the plants were harvested from the central Sudanese region of the Wight Nile, in the Kenana district. Following a thorough separation and cleaning process using distilled water, the coats and seeds were ground into a coarse powder using an electric grinder. Dry matter, ether extract, crude protein, crude fiber, and ash were measured from the coats. Using a grinding machine, The plant seeds were washed, dried in the shade, and pulverized into a powder.. Each ground sample was weighed and kept at room temperature in a dry container.

After the seed was ground into powder, its chemical makeup was examined.

samples Preparation:

Various instruments were used to determine the amounts of Ca, Fe, Zn, P, Cd, Mn, Cr, K, and Mg in the various plant components after all samples underwent chemical treatment. The parts of the solanum plant were dried, then coarsely ground in a clay mortar and sieved. The powder is kept in bottles to be used in the next procedures.

The chemical composition of the seed was investigated once it had been pulverized into a powder.

Sample preparation for AAS:

The solanum plant's components were dried, gently pulverized in a clay mortar, and sieved. Ten milliliters of concentrated $HClO_4$ were added to the mixture after 0.5 grams of the sample and 6 milliliters of (1:5) perchloric and nitric acid were added. The mixture was then left to stand at room temperature for the duration of the reaction. After tightly stopping the Teflon beaker to allow the acid vapors to escape, the beaker was heated in a sand bath at 200–250 °C for at least 6 hours, or until a white or non-turbid solution was the result of full digestion.

After that, the solution evaporated until it was either semi-dry or dry. The residue was put into a 50 ml volumetric flask and diluted with distilled water. To have the prepared solution ready for use for AAS within 30 days, it was put into a 50 ml plastic bottle and kept at room temperature.

***Solanum dubium* seed extract**

After shaking ten grams of crushed seed with one hundred milliliters of distilled water, the mixture was homogenized for fifteen minutes by an agitator. Twenty milliliters of the seed extract were utilized for analysis after it was filtered and refrigerated at 4ºC for a full day.

Methods for extracting Solanum dubium seeds:

The crude enzyme was extracted by the following methods: -

Freezing and evaporating under low pressure (freezing drying): Using distilled water, ground yellow fruits and seeds (100g each) were macerated for 24 hours, shaking intermittently during the first three hours. The solutions were then filtered. In order to remove the water, the filtrate was spread out over a shallow basin encircled by a freezing mixture and vacuum-sealed (13, 25, 10).

Distilled water extraction:

After shaking five grams of the crushed material for fifteen minutes at room temperature with thirty milliliters of distilled water, the mixture was filtered. The concentration and activity of the enzymes were measured using the aqueous filtrate (22, 25).

Making water extracts, methanol, and 96% ethanol

Using a shaker equipment, 500 grams of a sample containing a mixture of S. dubium seeds were extracted through maceration with 96% ethanol. Three days of extraction were spent, along with daily filtration. Using a rotary evaporator and lowered pressure, after mixing the filtrates, the solvent was removed. The same procedure was done with methanol (10, 13, and 21).

Characterization of solanum dubium seeds: -

Scan electron microscopy (SEM) :-

Samples of Solanum dubium were put into a JEOL JSM-6380LV SEM and placed on brass analysis stubs. The samples were processed and coated exactly as they were received. To avoid imaging issues, the microscope power was kept low, and pictures were captured under vacuum at different magnifications (figures 5, 6, and 7).

Fourier transform analysis

Using an FT-IR spectrometer (Therma Scientific), the mucilage's Fourier Transform Analysis (FTIR) spectra were obtained. After combining the dry powder with KBr, the mixture was mechanically compressed into pellets. The 4000–500/cm range was scanned to get the FT-IR spectra

III. Results and Discussion

The goal of the current investigation was to determine the metal content and examine the enzyme extract in Sudanese S. dubium seeds (27).

The contents of the Solanum dubium coat were 10.89% nitrogen-free extract, 5.78% fat, 97.05% dry matter, 12% crude protein, and 57.9% crude fiber. The following components made up the chemical composition of black cumin: 19.12% fat, 15.30% nitrogen free extract, ash (3.50%), 95.32% crude protein dry matter, and 27.25% crude fiber.

Table 1 shows the Solanum dubium enzyme's substrate specificity for several peptide substrates.

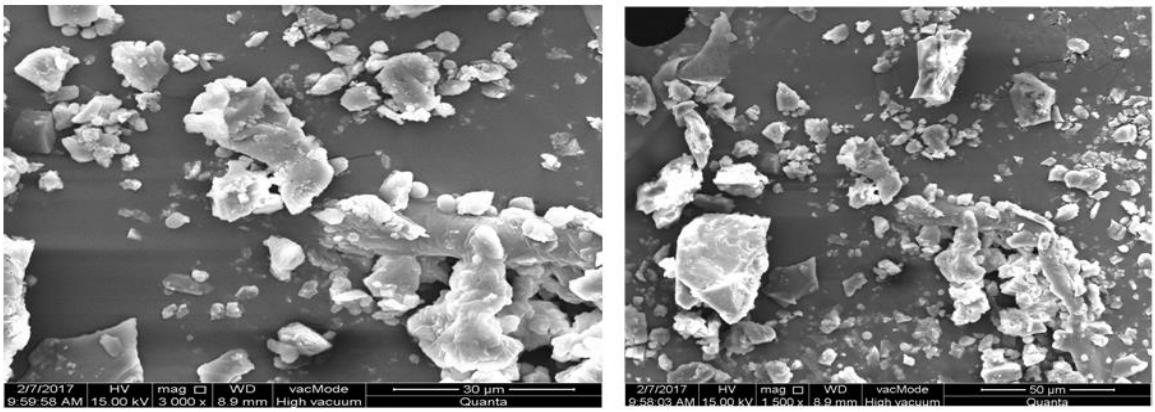
Substrate	substrate concentration (mM)	Km (mM)	Kcat (s^{-1})	Kcat/Km ($M^{-1} s^{-1}$)
N-Suc-Ala-Ala-Pro-Phe-pNA	2.0–0.26	10^{+4} 1.54	6.8 - 10 $1.9 - 10^{+2}$	4.89_ 3.96
Leu-pNA	N-Suc-Ala-Ala-Pro- 2.0–0.26	_ 10^{+4}	5.02 $4.2 - 10^{+2}$	3.08
	Ac-Tyr-Val-Ala- AsppNA	_ 10+5 1.01 – 0.25	1.40	
N-Suc-Ala-Ala	2.0 – 0.24	0 AlapNA Figure 5	0	0

Scan electron microscopy (SEM): -

Figures 5, 6, and 7 show various magnifications of the mucilage gathered from scanning electron microphotographs (SEM). Mucilage microphotographs indicate the possibility of an amorphous material.

The majority of the particles are observed as fibrous aggregates with erratic sizes and forms. The current study's SEM results indicate that the surface characteristic of mucilage affects its ability to retain water. According to reports, the extraction, purification, and product preparation processes can have an impact on the mucilage's shape, structure, and surface topography. Particle size and specific surface area have been shown to affect

solanum's hydration behavior, which in turn affects the substance's both molecular mass and intrinsic mass. Additionally, they found that particle size has an impact on the solanum's molecular mass and hydration kinetics.



Figures 5 and 6 show a high vacuum SEM of solanum seeds.

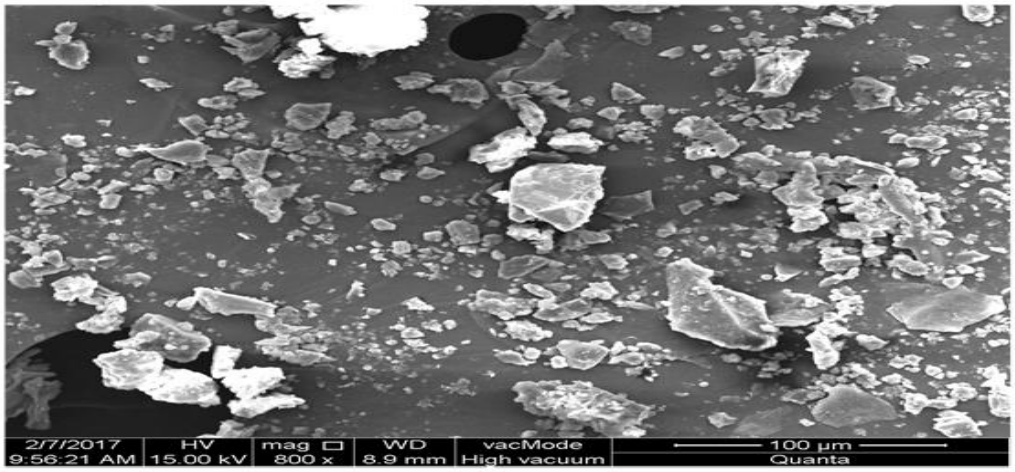


Figure 7: Seeds of Solanum Mucilage under various magnifications was examined using scanning electron microscopy with a Philips Lancashire XL-30 SEM.*3.2*

Infera red spectroscopy of solanum dubium

Fourier transform analysis software was utilized for the interpretation. The distinctive bands and peak of solanum were evident in the spectrum. The mucilage's spectra reveal a band at 2924.06 cm-1, which is caused by the presence of 2809 cm-1, which is caused by the alkyl C-H stretch or CH2 stretching modes. FTIR Spectrometry has been widely used to analyze the molecular and material structure of polymers.

Functional groups and the ways in which they cling to the polymer backbone are frequently identified using FTIR spectroscopic characterization (Baxter et al., 1992). The distinctive bands and peak of mucilage are visible in the FTIR spectra. Figure 8 displays the mucilage's FT-IR spectrum. "CHEMIX" O methylene C-H stretch (-CH2-) in school. The alkenyl C=N and C=C stretch's stretching mode is responsible for the peak observed at 1635.24 cm-1. CH3, or C-H methyl rock, is represented by absorption bands that lie between 1635.24 to 1379.30 cm-1. Additionally, the absorption C=O finding peaks at 1745.14 cm-1. Band 1160.80 is home to the CO or S=O break. In contrast to the C=C band 563.50, the band 720.16 CH2 is rocking.

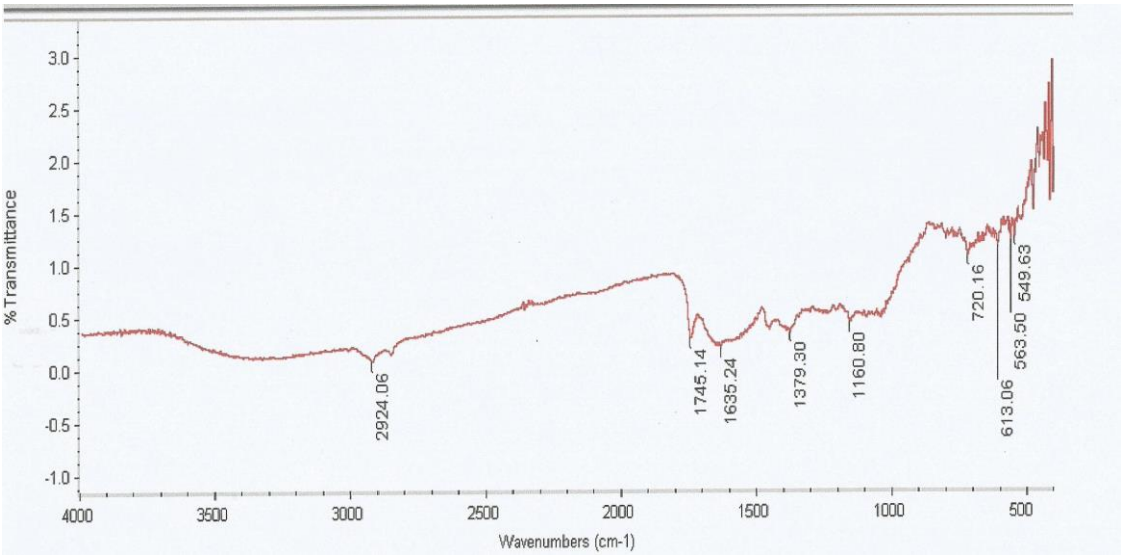


Figure 8: Solanium dubium infera red spectroscopy

results of metals analysis:

The amount of metal in solanium is determined by the characteristics of the soil, the surrounding environment, and user needs (fertilizer and pesticide use). Potassium and calcium are taken up by Solanum plants from the soil and concentrated in their leaves. Because of this, the number of metals in dobium varies greatly between nations.

This study measured the amounts of basic elements including Ca, Mg, K, and P, as well as Mn, Cr, Cd, Zn, and Fe, in solanum seeds. The sample revealed that K was more concentrated than any other metal flux, whereas solanum seed samples from the Kenana region of the Wight Nile had higher concentrations of Ca, Cr, Fe, Cd, Zn, and P.

Tables 2 and 3 exhibit metal concentrations detected in solanum seeds. The results of the investigation demonstrated a strong positive link between the amount of metals, especially heavy metals, in solanum.

Metal content in solanum was shown to significantly positively correlate. These findings raise the hypothesis that the higher metal concentration in solanum is related to the source's origin.

Table 2 shows the metal content in solanum dubium measure by atomic absorption.

Element	concentration mg/g
Ca	63.71
Mg	4130.0
Mn	0.3071
Cr	0.5287
Cd	-0.2470
Zn	21.6930
Fe	104.6580
K	582.0 (ppm)
P	12.0 (ppm)

Table (4)

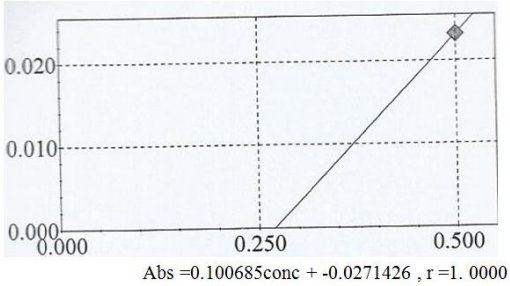
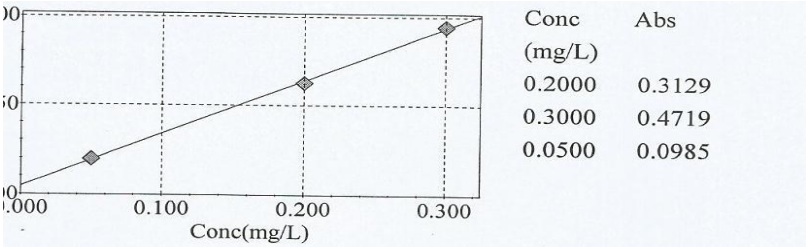
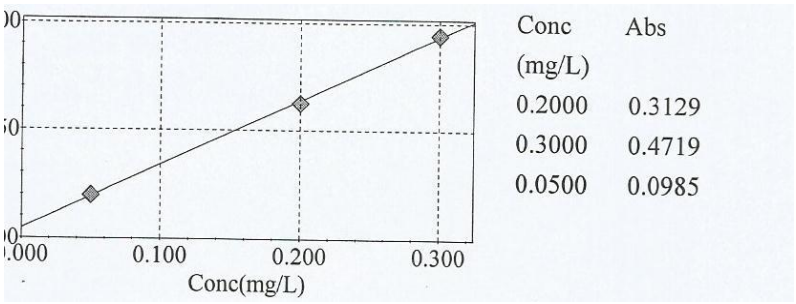


Figure (9) shows the calcium concentration calibration curve for solanum dubium.



r = 0.9996, Abs = 1.48853 conc + 0.0215368

Figure (10) shows the solanum dubium magnesium concentration calibration curve.



Abs = 0.9922, r = 0.9992, conc = 0.9353595,

Figure (11), manganese content calibration curve in solanum dubium

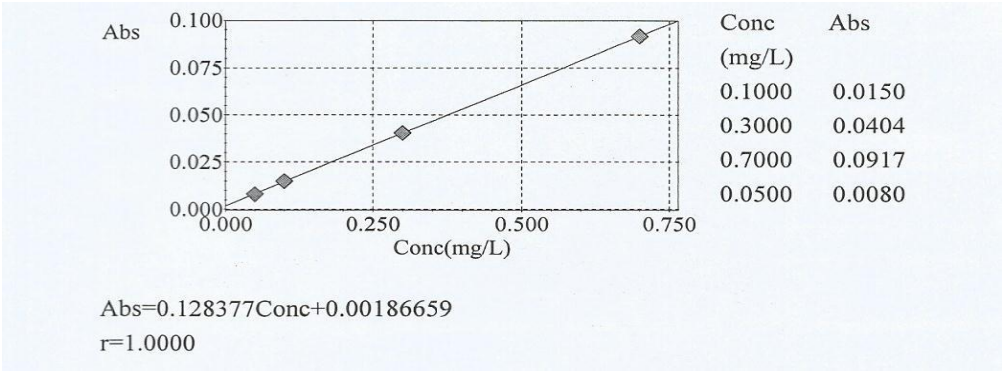


Figure (12) shows the calibration curve for the concentration of cadmium in solanum dubium.

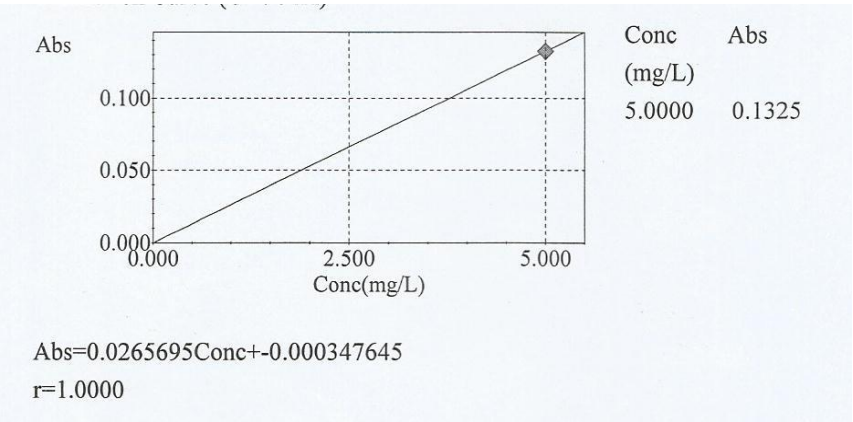


Figure (13) shows the solanum dubium chromium concentration calibration curve.

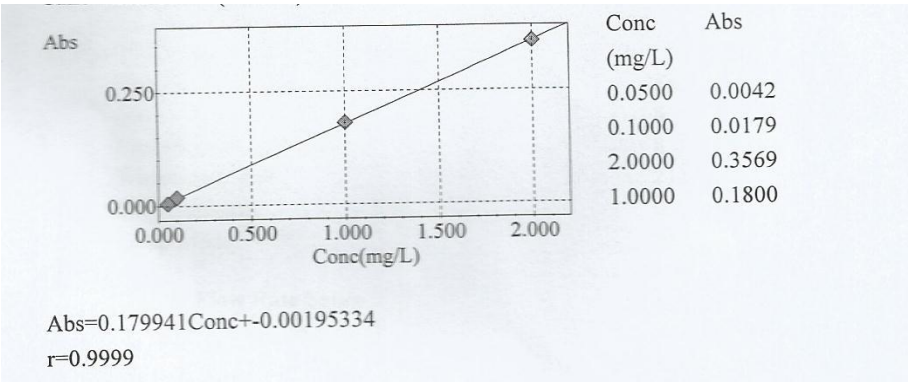


Figure (14) shows the zinc concentration calibration curve for solanum dubium.

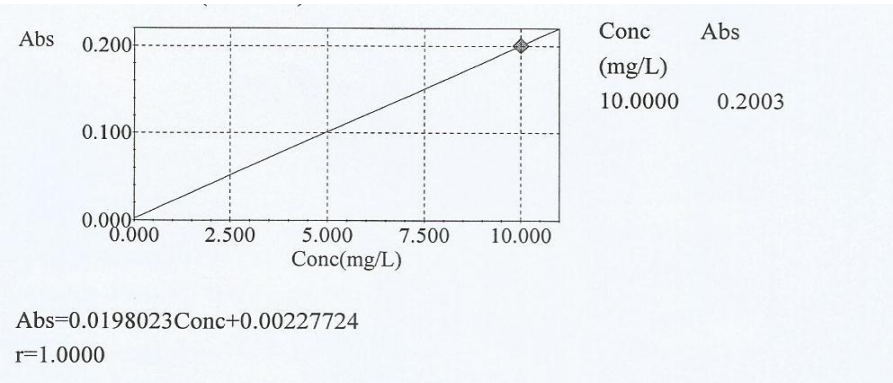


Figure (15) shows the ferric concentration calibration curve in solanum dubium.

The stability and activity of the isolated enzyme are affected by pH and temperature.

(a) The pure enzyme's pH stability. The enzyme (12µg) was incubated for one or twenty-five hours at 37°C at different pH values. Remaining activity was measured at pH 8.0 and 30°C after 40 minutes. (b) The effect of pH on the activity of the isolated protease (14). The enzyme activity was tested at various pH ranges of 5.0–12.8 at 30°C for 30 minutes using 1% azocasein as the substrate. 50 mM glycine-NaOH for pH 8.0–12.5, 50 mM Tris-HCl for pH 6.0–8.0, and 50 mM acetate/phosphate for pH 5.0–6.0 was the buffer that was employed.

How pH affects the activity of partially purified enzymes

As Fig. (16) shows, the isolated enzyme is stable across a wide pH range; it continued to function correctly in the pH range where dubium seeds are employed for milk coagulation. But to obtain an extremely pure enzyme, further purification is needed (11, 14).

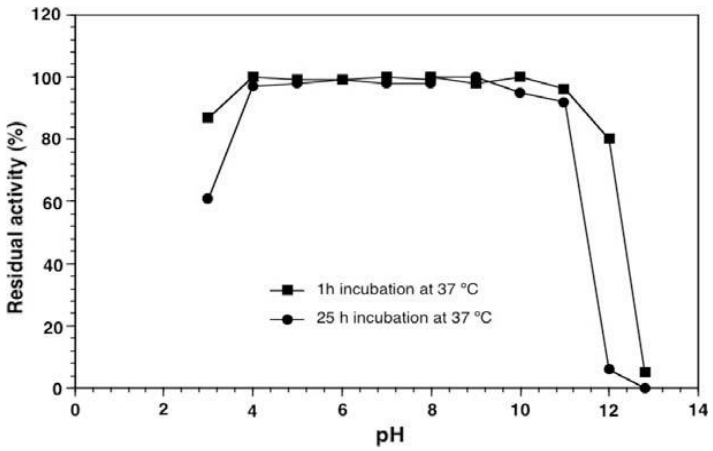


Figure (16): The pH has an impact on the stability of an enzyme that has been partially isolated from Solanum dubium seeds.6. How temperature affects the activity of partially purified enzymes

The findings displayed in Figure 16 demonstrated that when the temperature rose from 20 to 70 _C, the enzyme activity increased as well. At 70 _C, the activity was five and ten times greater, respectively., than at 40 and 20 _C. The moment temperature surpassed 80 _C, the activity quickly decreased. The stability of the enzyme at temperatures between 20 and 90 _C was investigated because of its high optimum temperature. After the enzyme was incubated for one hour at 60 degrees Celsius, all of its activity was kept, and after another hour at 70 degrees Celsius, roughly 70% of its activity was retained. It was discovered that the enzyme was thermostabilized up to 70 _C.

The temperature profile of the enzyme was shown to be comparable to plant serine proteases, which were found to resemble subtilisin/cucumisin by Asif-Ullah et al. (2006), Yamagata et al. (1989), and Uchikoba et al. (1990).

Compared to previous purification methods that have already been published, we concluded that this work has established a straightforward purification process to extract a very stable and active enzyme from S. dubium seeds for milk clotting. However, additional purification is required to get an enzyme that is incredibly pure (11, 14, 27).

The stability and activity of enzymes

Coagulants should not be sensitive to changes in the pH and composition of milk, since using rennet that is highly sensitive to pH changes could result in reduced yields and faulty cheese due to soft coagulants during cutting (Harboe and Budtz, 1999). The optimal pH for S. dubium's milk-clotting enzyme is 11.0, although it remains stable within a range of 4.0–11.0 (Fig. 17).

Compared to all the Cucumisin-like serine proteases identified in Cucumis trigonus Roxburghi, Cucumis melo L. var. Prince, Euphorbia milii, and trichosantus kirrilowi A, the isolated enzyme is more stable at basic pH (Asif-Ullah et al., 2005; Yamagata et al., 1989; Yadav et al., 2006; and Uchikoba et al., 1990).

These qualities are critical since, as noted by Lamas et al. (2001), most enzymes lose their catalytic stability at alkaline pH values, which restricts their applicability as coagulants in the production of cheese (20, 27).

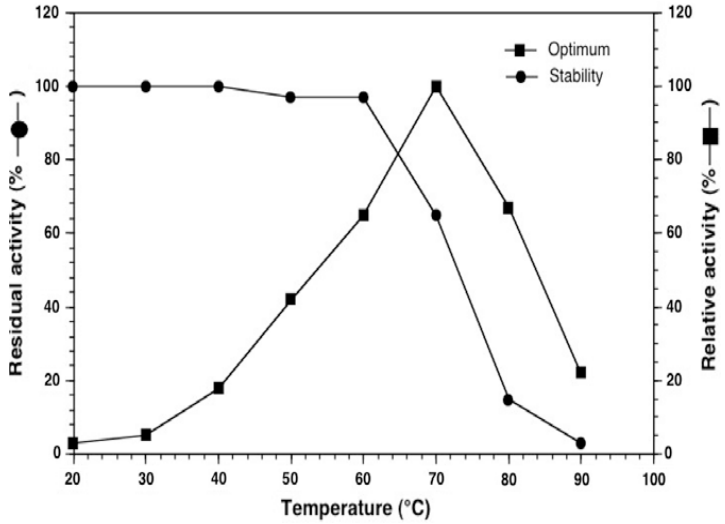


Figure 17. Temperature's effects on an enzyme's stability and activity in partially purified Solanum dubium seeds.

IV. Conclusions

The study's findings show that solanum seeds have higher concentrations of basic elements like Ca, Mg, K, and P as well as minerals like Mn, Cr, Cd, Zn, and Fe than are permitted. The maximum levels permitted for other metal flow are K and Ca, Cr, Fe, Cd, Zn, and P, in that order. A solanum seed sample was gathered from the Kenana region in the Wight Nile. Tables 2 and 3 exhibit metal concentrations detected in solanum seeds. The results of the investigation demonstrated a strong positive link between the amount of metals, especially heavy metals, in solanum.

The results demonstrate that none of the chemical components under investigation were significantly impacted by the coagulant type used in the solanum used in the cheese business. But while using powdered solanium seeds in the cheese industry, we need to exercise caution and utilize a little quantity of weight.

Higher activity was obtained from the enzyme extraction process using distilled water, however lower activity was obtained from longer soaking times. Activity rises as the fruit turns a dry yellow color. Up to 80°C

for milk can increase activity; up to 80°C for preheating can decrease activity; and up to 80°C for incubation and 1 ml of extract volume can increase activity.

For those who can use it in many dairy industries, the solanum extract enzyme is a good substitute enzyme that is also reasonably priced. It was suggested by the researcher to carry out more research in Solanum dubium in order to identify more active compounds and to create techniques and methods for purifying and separating the seed from the coat. to learn the solanum dubium in enzyme's structure.

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