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Seed surface characteristics and preliminary phytochemical analysis of *Coriandrum sativum* Linn. seeds of Apiaceae (Umbelliferae)

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ABSTRACT

For the seed surface characteristics the seed coat study is essential .The *Coriandrum sativum* L. seeds of Apiaceae (Umbelliferae) were studied for morphological, anatomical and phytochemical observations. The seeds of *Coriandrum sativum* L.shows thin, yellowish brown seed coat with well developed ridges and cellular networking. Anatomically the transverse section of seed coat shows epidermal layer with parenchyma, elongated scleroid fibrous layer with endosperm. The preliminary phytochemical analysis in different extract carbohydrates, terpenoids, reducing sugars, volatile oil or essential oil occure in more quantity. The qualitative tests were done for the detection of various chemical compounds. Medicinally it is very important. It is used for preparation of various drugs. Seeds are mostly used as a remedy on various diseases and disorders and also used in cooking purpose. The seed powder is used in spices. The plant is also useful as in vegetable. The morphological, anatomical, biochemical and phytochemical analysis of seed helps to determine micromorphological characters, drugs preparation. So it helps to solve many taxonomic problems and theruptic efficacy.

KEY WORDS :- Seed morphology, Scanning electron microscopy (SEM),Seed anatomy, biochemical, phytochemical analysis, Umbelliferae (Apiaceae)

INTRODUCTION :-

Seed is a mature ovule and a small embryonic plant enclosed in a covering called seed coat and usually with some stored food. *Coriandrum sativum* L. is an annual, herbaceous plant that grows 25 to 60 cm in height. It has thin, spindle-shaped roots, erect stalk, alternate leaves , and small, pinkish-white flowers(Burdock and Carabin,2009). For study of seed coat characters all the parameters are very important like seed size, shape, colour, hilum shape, hilum size, surface features, seed weight etc. The scanning electron microscopy is a modern technique for surface identification. Seeds have also the medicinal value. Seeds are evolved as a unique structural and functional entity to face the challenges imposed by changing environmental conditions. Seeds have acquired diversification in both external and internal characteristics so much so that each can be specified independently with definite set of characters. Seed coat surface show structural marking of great diversity.The plant is grown widely all over the world for seed, as a spice, or for essential oil production (Bhuiyan *et al*., 2009). Martin and Burkley (1973) indicated the ability to recognize seeds as an important diagnostic feature in modern agriculture, as without it, there will be little merit in perfecting methods of growing useful plant. Seeds are useful economically for increasing the trade and marketing industry. Baldwin (1942) uses tree seeds as potential sources of vegetable, fats, oils and chemicals or drugs. Tree fruits and seeds are also sources of drugs, dyes and other chemicals. The seed oil contained linalool and geranyl acetate (Asgarpanah. J.*et.al*. 2012).

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Fig-01 **Fig-02** **Fig-03**

Fig:-01- Habit of *Coriandrum sativum* L.
Fig:-02- inflorescence of *Coriandrum sativum* L.
Fig:-03- Fruits of *Coriandrum sativum* L.

MATERIALS AND METHODS:-

The present study deals with the investigation of seeds through various ways. The *Coriandrum sativum* L. seeds sample were collected from Barsayya seeds, Amravati. Voucher collections of the seeds examined are kept in the Department of Botany Model Art, Commerce & Science College Karanja (gh.) Dist-Wardha. Seed size, shape, colour, outline, position and shape of hilum and testa pattern were studied under disecting microscope and binocular microscope. Digital weighing balance was used for weighing the seeds in mg. The average weight of 15 seeds was taken. The morphological observations of seeds were done followed by their photography, using 1 cm. scale. This species of Apiaceae (Umbelliferae) were investigated for spermoderm ornamentation.

Scanning electron microscopy is most important for micromorphological investigation of seeds. For this purpose, the individual seeds were dipped in alcohol for 5-10 min, to remove the dust from them. The seed mounted on pin- type stubs using double sided adhesive tape or conductive silver paint to prevent charging of the surface during scanning and then coated with a very thin layer of gold in a polaron sputter coating unit. For spermoderm study of seed photomicrographs were taken in the Scanning Electron Microscope (LEO 430) at Birbal Sahni Institute of Paleobotany, Lucknow. *Coriandrum sativum* L. seed species were studied for their anatomical details. Seed coat anatomy is necessary for identification of anatomical details and differentiation of seed coat. Thin sections of seed coat or seed were taken and subjected to double staining procedure.Microphotography of thin sections was done on sophisticated trinocular microscope (Carl-Zeiss, Germany).

Qualitative test:

Qualitative test were done for the detection of protein, amino acids. For detection of amino acids using thin layer chromatography technique (TLC).

Test for protein : For Protein test the *Coriandrum sativum* L. seed sample were used for detection of proteins. For this process Biuret and Xanthoproteic test were done.

Precipitation reactions of proteins:- Proteins are precipitated from solution by heavy metal ions,($AgNO_3$,$CuSO_4$,lead acetate, mercuric chloride), alkaloidal reagents (trichloroacetic acid (TCA),Picric acid, meta-phosphoric acid) and concentrated salt solutions

$(NH_4)_2SO_4$,Na_2SO_4,NaCl).The precipitation os the result of the destabilization of protein-solvent interaction.

(i) Precipitation by heavy metal ions:- Place about 2ml of test solution and a few drops of Na_2CO_3 solution in four test tubes. To one add $AgNO_3$ solution dropwise: to the second $CuSO_4$;to the third $HgCl_2$ and to the fourth lead acetate .Note the formation of precipitate. Continue addition of excess salt solution with shaking and observe the dissolution of the precipitate.

(ii) Precipitation by alkaloidal reagents:- To 1 to 2 ml of test solution add 5-6 drops of the acidic reagent. Note the formation of precipitate. Divide the precipitate into 2 portions. To one portion add excess acid reagent. To the other portion add dil. NaOH. Observe the results.

(iii) Precipitation by concentrated salt solutions:- To about 3 ml test solution add saturated $(NH_4)_2SO_4$ solution with shaking . Note the formation of the precipitate.

(iv) Precipitation by organic solvents:- To 2 ml of test solution in two test-tubes, add ethanol and acetone to the two test-tubes and observe the results.

Reagents:-

1) Copper sulphate solution:- 1% $CuSO_4.5H_2O$ in water.
2) 10% (w/v) NaOH solution:- Dissolve 10g NaOH in 100 ml water.
3) Sodium carbonate solution:- 1% Na_2CO_3 in water.
4) Heavy metal salt solutions:- Prepare0.1Msolutionsof $AgNO_3$,$CuSO_4$, $HgCl_2$ and lead acetate in water.
5) Alkaloidal solutions:- Prepare 10% (w/v) aqueous solutions of TCA, picric acid and metaphosphoric acid in water.
6) Saturated ammonium sulphate solution:- Dissolve 76g of $(NH_4)_2SO_4$ in 100ml water at room temperature.
7) 95% Ethanol 8) Acetone.

Thin layer chromatography technique (TLC) of seed:

It is highly useful in research laboratories to separate, identify and characterize unknown compounds. A variety of small molecules like amino acids are separated by TLC technique. (Sadasivam and Manickam 2005)

Requirements :

1) Alumina plate of Merck with silica gel (20x20 cm),
2) Glass tank with lid.,
3) Developing solvent,
4) Sample (seed powder) (should be extracted following the procedures indicated for each group of compounds. For eg: extraction with 80% alcohol for amino acids.)
5) Standards,
6)Spraying agent (This also differs as for the group of compounds of interest.)

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Spray reagents : For amino acid : (Ninhydrin reagent) 100 mg. Ninhydrin to be dissolved in 100 ml. acetone.

Procedure for amino acid :

The *Coriandrum sativum* L. seed sample were studied for this purpose. Alumina plate MERCK (Germany) with silica gel 60 F_{254} (20 x 20 cm or 20 x 5 cm or 20 x 10 cm) was used for TLC. standard chromatogram using 24 known amino acids (each sample prepared in distilled water) for identification. The colour characters and R_f values were used to identify the amino acids present in the samples. The seed powder treated with 80% alcohol was used at the time of loading on plate. For this purpose 2-3 cm were distance from base of plate and marked the loading point. After loading the seed samples they were dried and placed in a glass tank covered with lid. Glass tank contained solvent n-butanol-acetic acid-water (80:80:20). After running the solvent the reading were noted and dried. Then sprayed with ninhydrin. The coloured spots were developed by heating the plates at 110^0C for 10 min. The R_f values of seed samples were matched with standard chromatogram and identified the amino acids by using R_f formula. The qualitative analysis through TLC is considered to be most effective, and useful method for the separation and identification of complex mixtures of herbal drugs (Wagner and Bladt, 1996)

Qualitative tests for carbohydrates:-

Methods

1. **Molisch's test :** To 1-2ml of test solution in a test tube, add 2 drops of Molisch's reagent. Mix well. Add 2-3ml of conc.H_2SO_4 along the sides of the test tube so as to form a layer below the carbohydrate solution. A purple ring appears at the interface. This indicates a positive reaction.
2. **Fehling's test :** To 2ml of Fehling's reagent in a test tube, add a few drops of test sample and boil in a water bath. The formation of a rusty brown color or red precipitate indicates a positive reaction.
3. **Benedict's test :** To about 0.5ml of test solution, add 2ml Benedict's reagent. Mix and place the test tube in a boiling water bath for 3-5min.The formation of a green, yellow, brown or red precipitate indicates a positive test for reducing sugars.
4. **Bial's test :** To 1ml of the test solution add 3ml Bial's reagent. Boil for a minute directly over a flame. Appearance of a green color is a positive test for pentoses.
5. **Seliwanoff's test :** Add 1ml of fructose solution to 5ml of Seliwanoff's reagent. Heat in a boiling water bath for about 30 sec. A red or pink color confirms the presence of fructose. Repeat the test with sucrose and glucose. Sucrose also answers the test rapidly while glucose may give a color after about 2-3 min.
6. **Iodine test :** Add 2 drops of iodine solution to 1ml of test solution in a test tube and observe the color formed. Starch gives a blue color while glycogen gives a reddish brown color.

Reagents

Molisch's reagents : Dissolve 50g α-naphthol in 100ml of alcohol (prepare fresh).

Benedict's reagent: Dissolve 173g of sodium citrate and 100g sodium carbonate in about 800ml water (heat, if necessary).Dissolve 17.3g$CuSO_4.5H_2O$ in about 100ml water and add to the carbonate-citrate solution with constant stirring. Make up to 1 liter in a measuring cylinder. Filter if required.

Fehling's reagent: Dissolve 7.0g of $CuSO_4.7H_2O$ in water and make up to 100ml.In a separate container, dissolve 24g of KOH and 34.6g of sodium potassium tartarate in water and make up to 100ml. Just prior to use, mix both the solutions.
Bial's reagent: Dissolve 1.5g of orcinol in 500ml conc. HCl and add 1.5ml of 10% ferric chloride solution.
Seliwanoff's reagent: Dissolve 0.5g of resorcinol in 1 liter of 1:2 diluted HCl. Iodine solution: Dissolve about 0.4g of iodine in 500ml of 3 % KI solution.

QUALITATIVE TESTS FOR LIPIDS:-
Method
1. Solubility test:- Place a few drops of test solution in a test tube and add 1-2ml of organic solvent (acetone or alcohol or benzene).Shake well. Repeat the test using water as the solvent.
2 Formation of a translucent spot on paper :- Place one drop of test solution on a piece of filter paper and observe the formation of a translucent spot which indicates a positive test.
3.Litmus test:- Dip blue litmus paper in a test solution and conclude the result..Blue litmus turns red.
4. Saponification:- Place a small quantity of test solution in a test tube and add 10ml of 10% alcoholic KOH solution. Boil the mixture for 10-15 min. or until the saponification is complete. To the hot solution slowly add Conc. HCl until the mixture is acidic. The free fatty acids formed will rise to the top as a clear oily layer. Cool the solution. The fatty acids will solidify and form a cake.
5.Test for unsaturation:-Take 1ml of the test solution in a test tube add bromine water to it drop by drop shaking after each addition until the bromine just fails to be decolorized.

Materials and reagents:-
1) Alcoholic KOH: Dissolve 10g KOH in 100 ml alcohol,
2) Bromine water: Add a few drops of bromine to 100 ml of water and shake.
3) Acetone.
4) Absolute alcohol.
5) Benzene.
6) Conc. HCl
7) Litmus paper.
8) Filter paper.

Preliminary phytochemical analysis:-
A) Collection & Identification of plant material:- Plant material (seeds) must be botanical identification based on the literature & placed in herbarium all data about the collection must be observed & documented.
B) Sampling of plant material:- The seeds of *Coriandrum sativum* L.collected from local places. Seeds were grinded in the mixer, prepare seed powder and kept in small plastic bags with proper labeling.
C) Preparation of aqueous extracts:- The seed powder of 5 gm. weighed using an electronic balance & 5gm of seed powder, then heat at 50-60◦c filter paper no.1 then filtrate was centrifuged at 2500 rpm for 15 minutes & the filtrate was collected in sterile bottles & was stored by refrigeration at 5^0C until use.

Preliminary phytochemicals analysis:-

This was carried out according to the methods described by Trease & Evans (1989).Qualification phytochemical analysis of the crude powder of the plants for the identification of phytochemicals like as a tannins, alkaloid, steroid, phenols & Terpenoid, Flavonoid etc.

Tannins:- (200 mg plant material+ 10 ml distilled water+ filtered) 2ml filtrate+ 2ml $FeCl_3$blue ------ Black precipitate indicate the presence of tannins & phenols.

Alkaloids:- (200 mg plant material+ 10ml methanol+ filtered) 2ml filterate+1%HCl+ steam 1ml filtrate+ 6 drops Mayer's reagent/Wagner's reagent/Dragendorff's reagent produced ---------- Creamish/Brown/red/orange precipitate indicate the presence of alkaloids.

Saponins:- 0.5ml filtrate+5ml distilled water --------- Frothing persistant indicate presence of saponins.

Terpenoids:- 2ml filtrate+ 2ml acetic anhydride+ Conc.H_2SO_4 -------- Green, blue precipitate indicates the presence of cardiac glycosides.

Steroids:- (Liebermann Burchard reaction):- (200 mg plant material+ 10ml chloroform+ filtered) 2ml filtrate+2ml acetic anhydride+ Conc.H_2SO_4 Blue, green ring indicate the presence of steroids.

Flavonoides:- (200 mg plant material+ 10ml ethanol+ filtered) -------- 2 ml filtrates+ Conc.HCl+ Magnesium ribbon ----------> Pink, tomato, red colour indicate the presence of flavonoids, glycoside.

Carbohydrates and glycosides:- Small quantity of extracts were dissolved separately in 5 ml of distilled water and filtered. The filterate was subjected to Molisch's test to detect the carbohydrates. Another small portion of extract was hydrolysed with dilute hydrochloric acid for few hours in a water bath and was subjected to Liebermann- Burchard's, legal and Borntrager's test to detect different glycosides.(pink to red color indicates presence of glycosides).
Protein:-Mellon's reaction:- Million's reagent:- (mercuric nitrate in nitric acid containing a trace of nitrous acid)usually yields a white precipitate on addition to a protein solution, which turns red on heating.

Volatile oil or essential oil:- A thick section of extract was placed on a glass slide. A drop of sudan red reagent was added and after two minutes, it was washed with 50% alcohol mount in glycerin.

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OBSERVATIONS:-

Fig:- 04

Fig:-05 - 43X

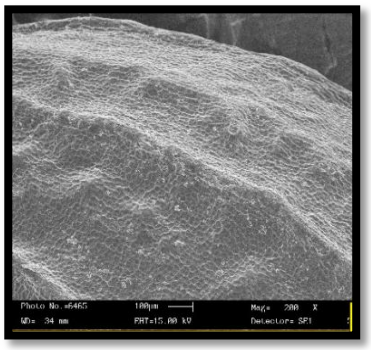

Fig:-06 – 200X

Fig:-04 – Seeds of *Coriandrum sativum* L**.**

Fig:-05 – Scanning electron microscopy of whole view of seed of *Coriandrum sativum* L. With prominent ridges and acute hilar region- 43X

Fig:-06 – Prominent ridges with mounded wavy and cellular networking-200X

The *Coriandrum sativum*(L.) commonly known as dhane/dhania/hara dhania/kothimbir belonging to family Apiaceae (Umbelliferae).Externally Seeds of *Coriandrum sativum* (L.) shows 0.27 cm. - 0.18 cm., ovate or spherical,yellowish,20.5 mg, bilateral**,** hilum apical, pointed, acute, seed shows prominent ridges on surface. It's a fruit like seed. Apical end acute. **(Fig-04)** The scanning electron microscopy of seed shows elongated ridges on the surface. The number of mounded irregularity present on surface. Surface shows cellular deposition. Cellular networking well developed on surface. Seed shows oval, globular in shape. Hilar region prominent towards apical end. On the surface number of ridges developed from apex to base. The ridges are wavy at some point. In between two ridges cellular deposition present which is pentagonal in nature. This surface shows number of irregular elongated mounded depositions on surface. **(Fig-5, 6)**

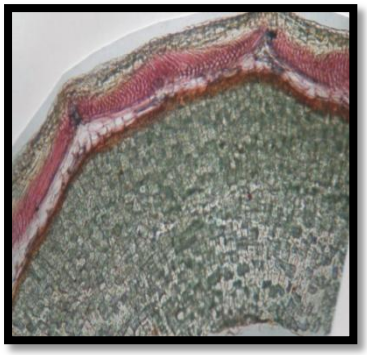

Fig:- 07 - 160X

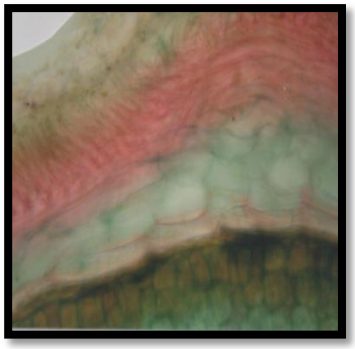

Fig:- 08 - 640X

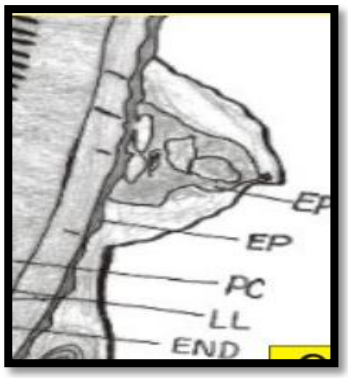

Fig:- 09 - 400X

Fig:-07-Outer epidermis wavy, parenchymatous cell with well developed endosperm of T.S. of seed coat -160X.

Fig:-08-Higher magnification view of seed coat shows pericarp, mesocarp and endocarp-640X.

Fig:-09- Sketched diagram of T.S. of seed coat shows outer wavy epidermis with cellular variations-400X

Internally the transverse section of seed coat shows outer most epidermis which is one layer and parenchymatous in nature. The seed internally divided into pericarp, mesocarp and endocarp. It's a fruit like seed which shows pericarp, seed coat and perisperm region. The perisperm is crystalloid form. Below epidermal layer sclerotic fibrous layer developed which is thick and wavy. The mesocarp is outer and inner layer. The endosperm and cotyledon well developed. The cotyledon cells measures 29.18µm in length and 11.10µm in breadth which is pentagonal shape. **(Fig-07, 08, 09)**

TABLE - 01 - Study of concentration of protein in seed samples

Sr. no	Biuret test	Xanthopro-teic test
01	++	++

TABLE:-02 – Qualitative test for *Coriandrum sativum* L. seed powder

Sr no.	Test	Observation	Coloration
01	Molisch's test (for carbohydrates)	----	Orange
02	Fehling's test(for carbohydrates)	++	Reddish brown
03	Bial's test(for carbohydrates)	+	Green
04	Seliwanoff's test(For Fructose) (for carbohydrates)	++	Red
05	Seliwanoff's test(For Sucrose) (for carbohydrates)	+	Pink
06	Test for Iodine	+++	Dark reddish brown
07	Benedict's test	----	Yellowish
08	TESTS FOR LIPIDS (Solubility test)	+ (water)	----
09	Formation of a translucent spot on paper	+	Faint yellow
10	Litmus test	+	Blue turns red
11	Test for Saponification	++ (Oily layer)	----
12	Test for Unsaturation	+	Decolorised

QULITATIVE TEST FOR PROTEIN

TABLE:-03 Precipitation reactions of proteins:- i) Precipitation by heavy metal ions

Sr no	AgNO3	CuSO4	HgCl2	Lead acetate
01	Reddish brown	Dark greenish	----	Milky white

TABLE:-04 (ii) Precipitation by alkaloidal reagents:- With picric acid

Sr no	Picric acid	NaOH
01	Yellowish	Brown

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TABLE:-05 (iii) Precipitation by concentrated salt solutions:-With NaCl

Sr no	Coloration
01	**Yellowish**

TABLE:-06 (iv) Precipitation by Organic Solvents

Sr no	Ethanol	Acetone
01	+	-

TABLE:- 07- Preliminary phytochemical observation of various extracts of seeds/ fruits of *Coriandrum sativum* L.

Sr no	Test for active constituents	Petroleum extracts	Chloroform extracts	Ethyl acetate extracts	Methanol extracts	Aqueous extracts
01	Alkaloids	----	----	----	----	+
02	Carbohydrates	+	+	+	----	+
03	Reducing sugars	+	----	+	+	+
04	Steroids	----	----	----	----	----
05	Glycosides	----	----	----	+	----
06	Flavonoids	----	----	----	----	----
07	Terpinoids	+	+	----	+	+
08	Saponine	----	----	+	----	----
09	Protein	+	----	----	+	+
10	Tannins	----	----	----	+	+
11	Amino acids	----	----	+	----	+
12	Volatile oil or essential oil	----	+	+	----	+

(+) indicate present, (--) indicate absent

Medicinal uses: Coriander fruit/seed used in urethritis, cystitis, urinary tract infection urticaria, rash, burns, sore throat, vomiting, indigestion, nosebleed, cough, allergies, hay fever, dizziness and amoebic dysentery. Dried seeds are the most common part used in cooking. (Pathak.N.*et al.* 2011). It has various health-related benefits and used in various Unani formulations (Singh. M *et.al* 2015) The seeds are used as a drug for indigestion, against worms, rheumatism, and pain in the joints (Wangensteen *et al.*, 2004).

Fig:- 10- Thin layer chromatography of *Coriandrum sativum* L .seed shows L-Cysteine hydrochloride, L-Leucine, Isoleucine, DL-Valine.

DISCUSSION :-

From the above observations the seed coat study shows morphological and anatomical variations in which the seed surface characters were well studied by scanning electron microscopy. The seed coat anatomy also helps for determination of seed characters. The biochemical and phytochemical analysis helps for detection of various chemical compounds present in seeds of *Coriandrum sativum* L. The complex chemical compound of herbal plants were detected by qualitative analysis.The compound like L-Cysteine hydrochloride, L-Leucine, Isoleucine, DL-Valine are found in thin layer chromatography technique. Detection of protein, lipids and carbohydrate and other are given by qualitative test. The complex compound were detected by preliminary phytochemical observations. Various extract like petroleum ether,chloroform,ethyl acetate,methanol and aqueous form were used for detection of active constituent. carbohydrate ,reducing sugars, terpenoids and proteins and volatile oil or essential oil were mostly present in these extract. So seed identification is an important feature for detection of micromorphological characters and detection of various chemical constituents. So seed identification both externally and internally provide various information and reliable criteria about unknown seeds. Seed shows high medicinal value, used in preparation of various drugs. Various chemical compositions present inside the seed are effective for drug preparation. *Coriandrum sativum* L. is a potent medicinal plant. It highlighting its traditional application as well as recent findings for the pharmacological and clinical applications. So identification, detection and analysis are most important for better research for various purposes and economic use also.

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