IJPSR (2018), Volume 9, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 06 October, 2017; received in revised form, 26 December, 2017; accepted, 30 December, 2017; published 01 July, 2018

COMPARATIVE PHARMACOGNOSTIC EVALUATION AND STANDARDIZATION OF *CAPSICUM ANNUUM* L. (RED CHILI)

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Keywords:

Capsicum annuum L., Pharmacognostic evaluation, Standardization, FT-IR, SEM-EDAX

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ABSTRACT: Present study includes the detailed pharmacognostic evaluation in terms of macroscopic and microscopic study along with physicochemical, phytochemical and fluorescence analysis of *Capsicum annuum* L., an annual spice and member of family Solanaceae, commonly known as red chili. The pharmacognostic studies provide comprehensive data not only in the identification but at the same time is an excellent tool for the evaluation of quality of spices. The Fourier Transform-Infrared spectrophotometer (FT-IR) analysis and elements detection by scanning electron microscope-energy dispersive x-ray spectroscopy (SEM-EDAX) was also performed and noted to be quite helpful in evaluation, authentication and judgement of quality of *C. annuum.* The scanning electron microscope-energy dispersive X-ray spectroscopy (SEM-EDAX) technique has been used in the present studies to detect the concentration of trace elements. Results of the present study indicated comprehensive information to check, evaluate and standardized commercial samples by the manufacturers and supplier with respect to purity, identity, safety, quality of red chili and other spices.

INTRODUCTION: *Capsicum annuum* L. is an annual spice, belongs to the family *Solanaceae* also known as bell pepper, green pepper, sweet pepper, cherry pepper and chili pepper. It is widely grown in Pakistan, America, Mexico, India, Indonesia, China, Ethiopia, Spain, Portugal, South Africa and Central Europe. The major components of red chili are capsaicin ($C_{18}H_{27}NO_3$) (trans-8 methyl-N-vanillyl-6-nonenamide) and dihydrocapsaicin (8 methyl - Nvanillylnonen amide). About twenty different pigments have also been identified including green chlorophylls, violaxanthin, yellowish orange lutein, antheraxanthin, *β*-cryptoxanthin, *β*-carotene and zeaxanthin.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(7).2807-17

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(7).2807-17

Red capsanthin, capsorubin, cryptoxanthin, ascorbic acid, thiamine and fixed oil also present in red chilli [1 - 3]. Nutritional compounds are also been reported by some authors, which include carbohydrates, protein. Apart from these, bioactive groups have also been identified and reported alkaloids, phenolic compounds, saponins, tannins and flavonoids *etc* [4].

In addition pro-Vitamin A and Vitamins B, C and E also present in red chilli [5]. From pharmacological point of view red chili possess analgesic, anti-carcinogenic, anti-coagulant, anti-microbial, anti-oxidant, anti-tumor, arteriosclerosis, bronchitis and carminative activities. It also effective in cough, otitis, increase blood circulation and in rheumatism [2 - 4, 6 - 9]. It has also many household applications such as natural coloring agent, as an ornamental plant, as an appetizer and in cosmetics, as well as pharmaceuticals (capsaicin cream) and in food manufacturing industries.

Red chili has pungent taste so it is used as a spice in different types of curries, mix with other spices in food preparation. It also used in making sauces, pickles, chutneys, rice, maize *etc*. [3, 7, 10]. The existing study object was to evaluate and standardize red chili samples in three different forms which were available in local market using pharmacognostic parameter in term of organoleptic, physicochemical analysis, fluorescence analysis, and phytochemical analysis as well as FTIR and SEM-EDAX methods. The obtained data of present study indicated that all parameters are highly useful for the correct identification and authentication of red chili.

MATERIALS AND METHODS:

Red Chili Samples: Three different form whole fruit red chili, flake red chilli and powdered red chili were purchased from local market of Karachi, Pakistan. Samples were identified and the specimen numbers were assigned specimen numbers (RC001, RC002 and RC003) respectively and deposited in the department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi.

Powder Preparation: The dried samples whole fruit and flake were grounded to get powder sample before use and stored in a dry and air tight container.

Extract Preparation: The extracts of all three test samples in ethanol, hexane, chloroform and distilled water were prepared according standard method [11].

Macroscopic Evaluation: To determine the macroscopic or organoleptic evaluation standard methods were used [12 - 14]. Macroscopic examination of test samples includes, by means of sense organs such as color, odor, taste, shape and size and other features like touch and texture *etc*.

Microscopic Evaluation: Small amount of powder test sample was taken on a glass slide and one drop of 5% iodine reagent was poured in it and cover slip was placed on slide. Different microscopic features of test sample was observed under fluorescence microscope and recognized the different tissues by microphotography. The same method was repeated with 5% glycerin and 5% chloral hydrate reagents. These three reagents iodine, glycerin, and chloral hydrate were applied for assessment of cork, fibers, stomata, pollen cells, trichomes and calcium oxalate, while for the confirmation of starch granules iodine reagent was used [15].

Physicochemical Analysis: All physicochemical analysis, such as foreign matter, loss on drying, total ash, acid-insoluble ash, water-soluble ash, alcohol soluble extractives (Hot extraction method), alcohol-soluble extractives (Cold extraction method), water-soluble extractive (Hot extraction method), water-soluble extractive (Cold extraction method) and crude fiber were performed according to the standard reported method [13].

Phytochemical Analysis:

Qualitative Phytochemical Analysis: Qualitative phytochemical analysis were performed as prescribed standards methods to determine the presence and absence of metabolites in test samples such as alkaloids (Mayor test), carbohydrates (Benedict's test), flavonoids (Shinoda test), saponins (froth test), protein and amino acids (Ninhydrin test) as described by Arora *et al*., 2013 [12], fixed oil and fats and cardiac glycosides (Borntrager's test) as described by Madhukar 2013 [16], tannins (Braymer's test) by Ugochukwu *et al*., 2013 [11] and coumarin test as described by Harsha *et al*., 2013 [17].

Fluorescence Analysis: Test sample was treated with distilled water, 1N HNO_3, glacial acetic acid, concentrated H_2SO_4 and with concentrated HCl, then fluorescence characteristics were examined under day and UV lights at the range of 254 nm and 366 nm [18].

FT-IR Analysis: Determination of functional group in test samples, analysis was performed through Fourier Transform - Infrared spectro photometer (FT-IR) and the method was performed as per reported standard procedure. Sample (10 mg) was placed on crystal of ZnSe (Zinc Selenide) crystal attached with ATR detector and applied about 8 units pressure and spectrum was collected. The FT-IR spectrum of sample was recorded between 400 to 4000 cm^{-1} [19].

Detection of Trace Elements:

Scanning Electron Microscope (SEM): SEM was performed to evaluate the powder surface

morphology and particle size using JSM 6380 A. Scanning electron microscope. For this purpose powder sample was placed on specimen stub with two sided adhesive tape and coated with thin layer of gold using quick auto coater model number JFC 1500. This process was done under a high vacuum, using 20 keV electron beam energy while the detector used with highest resolution with less energy. For the identification of trace element in tested sample EDAX detector (EX-54175 JMU) was used which attached to electron microscope [20, 21].

RESULTS AND DISCUSSION:

Pharmacognostic Evaluation:

Macroscopic Evaluation: Organoleptic evaluation (Macroscopic evaluation) is the first step for the identity and purity of spices and it should be carried out before performing any other test. Macroscopic examination of sample includes, visual examination by naked eye and following macroscopic evaluation has carried out such as color, odor, taste, shape and size and other features like touch and texture *etc* [12, 2]. The macroscopic evaluation results showed that color of fruit (Red-orange), flake (Yellowish red) and powder (bricks red) **Fig. 1 - 3**, odor characteristic, aromatic with intensely pungent and hot taste having oblong and conical shape with weakly wrinkled and glossy texture and size 70 - 100 mm long, 40 - 45 mm diameter (average = 80.6 mm long, 42.2 mm diameter). Results of whole fruit, flake and powder macroscopic evaluation showed in **Table 1**.

FIG. 1: *CAPSICUM ANNUUM* FRUIT

FIG. 2: *CAPSICUM ANNUUM* FLAKES

FIG. 3: *CAPSICUM ANNUUM* POWDER

TABLE 1: MACROSCOPIC STUDY OF *CAPSICUM ANNUUM* FRUIT

Characters	Sample 1	Sample 2	Sample 3
Color	Red, Orange	Yellowish red	Bricks red
Odour	Characteristic, aromatic	Characteristic, aromatic	Characteristic, aromatic
Taste	Intensely pungent	Intensely pungent, hot	Intensely pungent, hot
Size	70-100 mm long, 40-45 mm diameter	Not applicable	Not applicable
Shape	Oblong, conical	Not applicable	Not applicable
Texture	Weakly wrinkled and glossy	Not applicable	Not applicable

Microscopic Evaluation of Powder Red Chili: The purpose of microscopic evaluation is to identify the characters of plant tissues and to check the purity of sample. Microscopic evaluation is a good analytical technique and a quick way to check the contamination of different plant or material.

Information of the present study will provide data which is helpful in the correct identification and authentication of test sample and may help in preventing adulteration. The powder of red chili is reddish orange in color, strong characteristic aromatic odor withhotin taste.The microscopic evaluation of powdered red chili fruit has as shown in **Fig. 4 - 21**. Fiber, trichome, cluster calcium oxalates, sclereids of endocarp, epidermis cells, elongated sclereids, calcium oxalates and glandular trichome, endosperm cells, parenchyma of mesocarp with oil glands, group of vessels, annular vessel, fiber, mesocarp cells, epidermis of testa, oil globules, yellow fragment of epicarp, epicarp with cuticular striation and epicarp with stomata. During microscopic evaluation of powder, no significant differences were noted in the microscopic features of all the three samples. Therefore, **Fig. 4 - 21** may be treated as reference for all three samples.

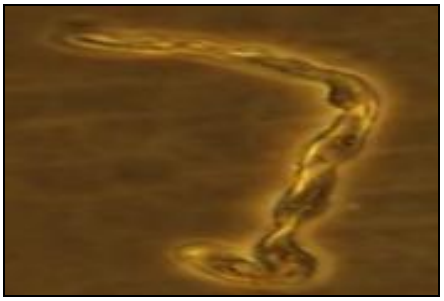

FIG. 4: FIBER

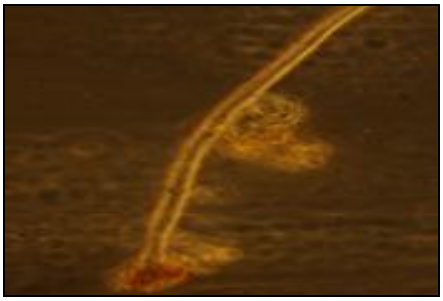

FIG. 5: TRICHOME

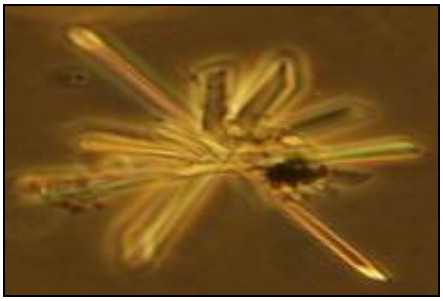

FIG. 6: CLUSTER CALCIUM OXALATES

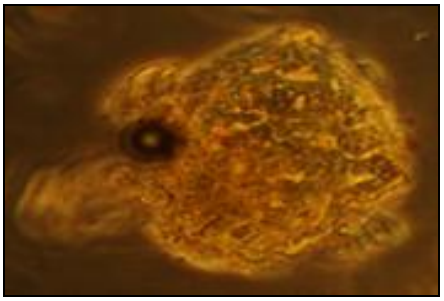

FIG. 7: SCLEREIDS OF ENDOCARP

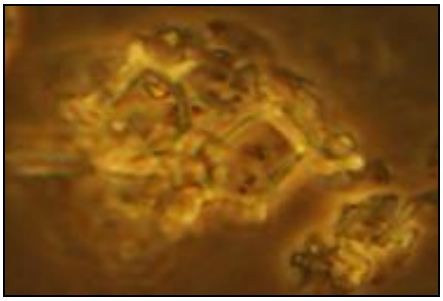

FIG. 8: EPIDERMIS CELLS

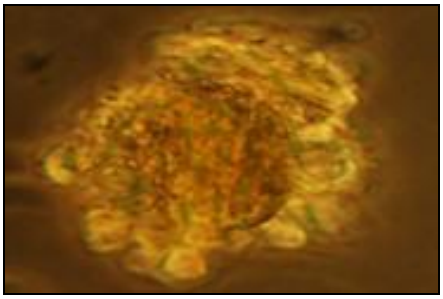

FIG. 9: ELONGATED SCLEREIDS

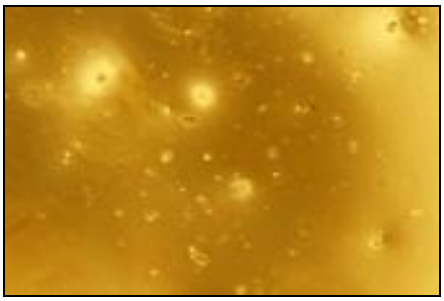

FIG. 10: CALCIUM OXALATES AND GLANDULAR TICHOME

FIG. 11: ENDOSPERM CELLS

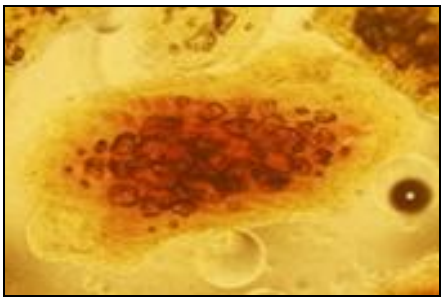

FIG. 12: PARENCHYMA OF MESOCARP WITH OIL GLANDS

TABLE 2: PHYSICOCHEMICAL ANALYSIS OF POWDER *CAPSICUM ANNUU*

Test	Standard limit (%)	Values		
		Sample 1	Sample 2	Sample 3
Foreign matter	NMT 1%	0.8% w/w	0.8% w/w	0.7% w/w
Loss on drying	NMT 11%	6.0% w/w	7.1% w/w	6.5% w/w
Total ash	NMT 8%	7.2% w/w	7.8% w/w	6.0% w/w
Acid-insoluble ash	NMT 1.3%	1.2% w/w	1.3% w/w	0.9% w/w
Water soluble ash	N.A	1.5% w/w	1.5% w/w	1.2% w/w
Hot extraction (Alcohol soluble)	N.A	7.3% mg/g	7.1% mg/g	7.7% mg/g
Cold extraction (Alcohol soluble)	N.A	12.9% mg/g	13.7% mg/g	13.9% mg/g
Hot extraction (Water soluble)	N.A	11.9% mg/g	11.1% mg/g	12.3% mg/g
Cold extraction (Water soluble)	N.A	9.9% mg/g	9.1% mg/g	10.9% mg/g
Crude fiber	N.A	40% w/w	38% w/w	41% w/w

Note: (NMT) Not more than, (N.A) Not available (limit)

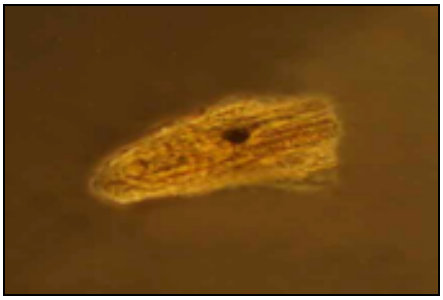

FIG. 13: GROUP OF VESSELS

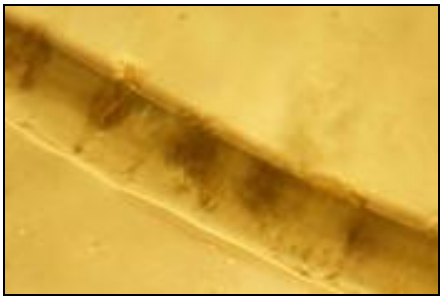

FIG. 14: ANNULAR VESSEL

FIG. 15: FIBER

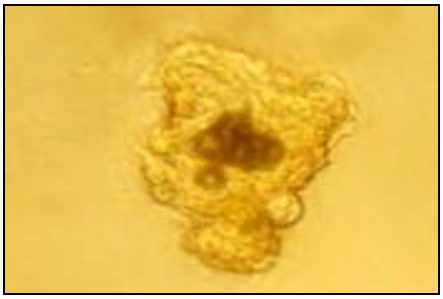

FIG. 16: MESOCARP CELLS

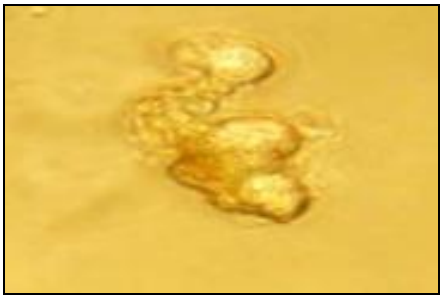

FIG. 17: EPIDERMIS OF TESTA

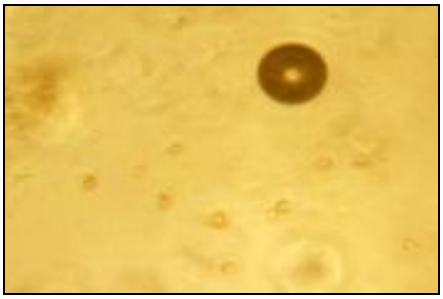

FIG. 18: OIL GLOBULE

FIG. 19: YELLOW FRAGMENT OF EPICARP

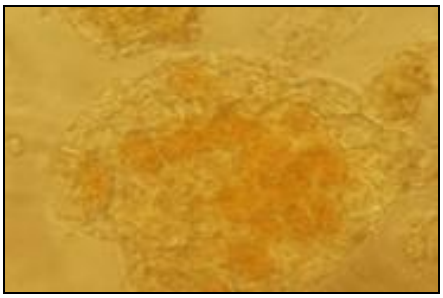

FIG. 20: EPICARP WITH CUTICULAR STRIATION

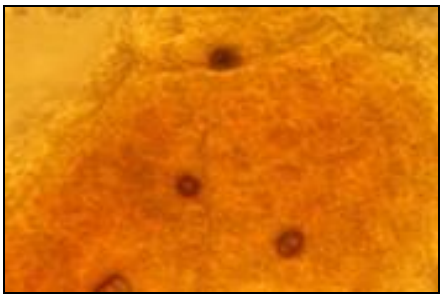

FIG. 21: EPICARP WITH STOMATA

Physicochemical Analysis: Physicochemical analysis results of all three test samples have been shown in **Table 2.** The respective tests were performed for the presence of foreign matter which were found 0.8%, 0. 8%, 0.7%, loss on drying 8.9%, 8.7%, 6.5%, total ash 7.2%, 7.8%, 6.0%, acid insoluble ash 1.2%, 1.3%, 0.9%, water soluble ash 1.5%, 1.5%, 1.2%, while alcohol soluble extractive hot and cold extraction were7.3%, 7.1% , 7.7% and 12.9%, 13.7%, 13.9% respectively. The water soluble extractive hot extraction were 11.9%, 11.1%, 12.3% water soluble cold extraction were 9.9%, 9.1%, 10.9% and crude fiber were found 40%, 38% and 41%. Physicochemical analysis is an important parameter for detecting the adulteration in medicinal plants.

Determination of foreign matters (insects, moulds, stone, soil, animals excreta) is useful in quality profile of a crude drug standardization and as a first step in physicochemical analysis the identification of the purity of crude drug may take place by this method it was found 0.87%, under limit prescribed by WHO, total ash value is very important for the determination of purity of drug. For example presence or absence of physiological ash (tissue) and non-physiological ash (foreign matter *i.e.* soil and sand) indicates the purity of spices [22]. The values of total ash in all three samples were found from 6.0% to 7.8% showing a high amount of organic matter.

Loss on drying determined the presence of moisture in sample, they were from 6.0% to 7.1% and the low percentage of moisture shows that there is no growth of microorganism. While acid insoluble ash values indicate the presence of inorganic materials and they were observed from 0.9% to 1.3%, low percentage indicating the less amount of in organic matters. Alcohol and water soluble extractives value indicated the concentration of chemical constituents in test sample. Crude fiber helpful in determination of difference between similar plant or detection of adulterants in sample [23].

Phytochemical Analysis: Phytochemical analysis of all three samples of red chilies were revealed in **Table 3**. Phytochemicals are natural bioactive constituents of plants these bioactive constituents not only used as a nutrient but also have medicinal values to protect against diseases and provide fitness of human health. These compounds also provide the protection to plants cells from insects and environmental hazards and as well as give taste, fragrance and color to plants. As well as provide disease preventing functions like anti- cancer, anti-oxidant, detoxifying agents, neuro pharmacological agents, immunity potentiating agents, decrease of platelets aggregation and modulation of hormone in human body.

TABLE 3: PHYTOCHEMICAL ANALYSIS OF METABOLITES

Metabolites	Ethanol extract			Hexane extract			Chloroform extract			Distilled water extract		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	+	+	+	+	+	+
Fixed oil and fats	+	+	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Coumarins	-	-	-	-	-	-	-	-	-	-	-	-

Note: Sample 1 (S1) Sample2 (S2) Sample 3 (S3), Present (+) Absent (-)

Present result of phytochemical analysis clearly showed that alkaloids, carbohydrates, flavonoids, saponins, proteins, fixed oil and fats, cardiac glycosides and tannins were present in all extracts of test samples while coumarins were not observed in any test samples.

Fluorescence Analysis: Fluorescence analysis was performed on powder red chili fruits sample (1), sample (2) and sample (3) are reported in **Table 4 – 6** respectively. As well as on extracts are presented in **Table 7 - 9** respectively.

TABLE 4: FLUORESCENCE ANALYSIS OF SAMPLE 1 IN POWDER FORM

Treatment	Under ordinary light	Under UV light	
		254 nm	365 nm
Powder as such	Orange	Brown	Dark red
Powder + Distilled Water	Orange	Greenish yellow	Black
Powder + H_2SO_4 (Conc.)	Blackish red	Dark brown	Grayish black
Powder + HCl (Conc.)	Reddish orange	Greenish brown	Dark brown
Powder + Glacial acetic acid	Orange	Greenish brown	Reddish brown
Powder + 1N HNO_3	Orange	Yellow	Brown

TABLE 5: FLUORESCENCE ANALYSIS OF SAMPLE 2 IN POWDER FORM

Treatment	Under ordinary light	Under UV light	
		254 nm	365 nm
Powder as such	Red	Brown	Dark red
Powder + Distilled Water	Red	Reddish green	Black
Powder + H_2SO_4 (Conc.)	Reddish orange	Greenish brown	Brown
Powder + HCl (Conc.)	Blackish red	Dark brown	Black
Powder + Glacial acetic acid	Reddish orange	Greenish brown	Reddish brown
Powder + 1N HNO_3	Dark red	Brown	Reddish brown

TABLE 6: FLUORESCENCE ANALYSIS OF SAMPLE 3 IN POWDER FORM

Treatment	Under ordinary light	Under UV light	
		254 nm	365 nm
Powder as such	Orange	Brown	Dark red
Powder + Distilled Water	Orange	Greenish yellow	Black
Powder + H_2SO_4 (Conc.)	Reddish yellow	Reddish brown	Grayish black
Powder + HCl (Conc.)	Black	Dark brown	Dark brown
Powder + Glacial acetic acid	Reddish orange	Greenish red	Reddish brown
Powder + 1N HNO_3	Reddish orange	Greenish yellow	Brown

TABLE 7: FLUORESCENCE ANALYSIS OF SAMPLE 1 IN EXTRACT FORM

Extract	Under ordinary light	Under UV light	
		254 nm	365 nm
Hexane	Deep red	Reddish brown	Reddish black
Ethanol	Deep red	Brown	Black
Chloroform	Deep red	Brown	Black
Distilled water	Red	Brown	Black

TABLE 8: FLUORESCENCE ANALYSIS OF SAMPLE 2 IN EXTRACT FORM

Extract	Under ordinary light	Under UV light	
		254 nm	365 nm
Hexane	Red	Brown	Reddish brown
Ethanol	Deep red	Brown	Reddish black
Chloroform	Deep red	Brown	Black
Distilled water	Red	Brown	Bluish black

TABLE 9: FLUORESCENCE ANALYSIS OF SAMPLE 3 IN EXTRACT FORM

Extract	Under ordinary light	Under UV light	
		254 nm	365 nm
Hexane	Deep red	Reddish brown	Reddish black
Ethanol	Deep red	Deep red	Black
Chloroform	Deep red	Brown	Reddish black
Distilled water	Red	Brown	Black

Fluorescence analysis is an important pharmacognostic tool for the identification of medicinal plants and gives the precise and satisfactory results without spending several dilution steps as other analysis of pharmaceuticals sample. Each phytochemical compound showed specific fluorescent coloration after the treatment of chemical reagent under UV light with 254 nm and 365 nm wave length, such as alkaloids showed reddish brown color, flavonoids showed yellow color, terpenoids showed reddish brown, glycosides showed reddish brown or bluish green color, sterol when treated with 50% H_2SO_4 showed yellowish green fluorescent. The results obtained from fluorescence analysis performed during the study, for the supported present study of phytochemical analysis for the presence of alkaloids, carbohydrates, flavonoids, protein, fixed oil and fats, tannins and cardiac glycosides in test samples.

FT-IR Analysis: The FTIR absorption spectrums of sample 1, 2 and 3 showed in **Fig. 22 - 24** respectively. FT-IR analysis is a time saving method to identify functional groups in test sample [24]. The FT-IR spectrum of all samples revealed that the peak arises in the range 3400 cm^{-1} to 1020 cm^{-1}. The test samples showed intense peaks from 3200 cm^{-1} to 3400 cm^{-1}due to the characteristic stretching vibration of N-H and O-H from alkaloids, polyphenols amino acids, while the absorption peaks from 2807 cm^{-1} to 2923 cm^{-1} were appeared from the C-H symmetric stretching of CH_3 and CH_2 group from carboxylic acids structure, the peaks from 1700 cm^{-1} to 1710 cm^{-1} were appeared from C=O stretch bending which indicated the presence of saponins, while the absorbance peaks from 1624 cm^{-1} to 1650 cm^{-1} from C=C stretch bending to indicated the presence of conjugated alkene group in all test samples.

The strong absorbance peaks from 1020 cm^{-1} to 1250 cm^{-1} were appeared in samples from C - O stretching vibration, indicated the presence of alcohol, ether and carboxylic acid **Table 10 - 12**.

All three FT-IR spectra of test samples are similar. The FT-IR analysis results confirmed the presence of aromatic amines, esters, hydroxyl, alkanes, phenol, alcohol, alkenes and alkynes compound in all test samples.

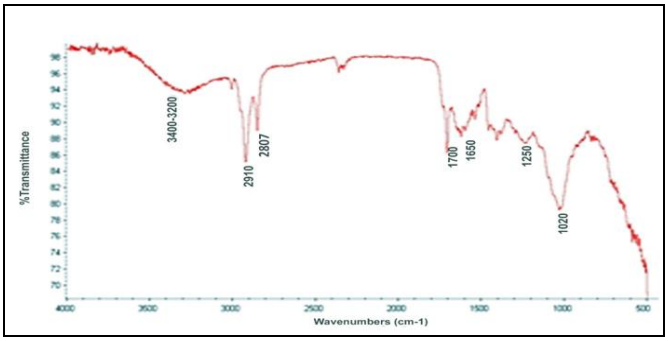

FIG. 22: THE FT-IR SPECTRUM OF *CAPSICUM ANNUUM* POWDER SAMPLE 1

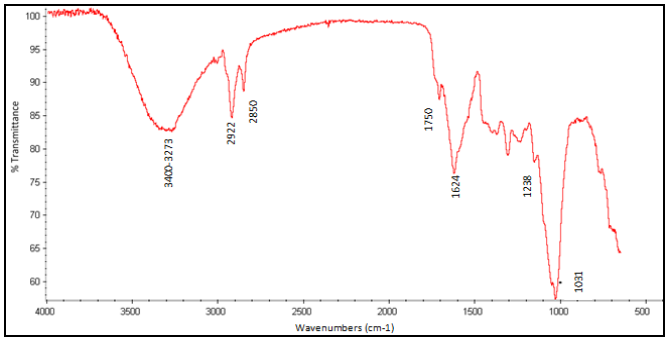

FIG. 23: THE FT-IR SPECTRUM OF *CAPSICUM ANNUUM* POWDER SAMPLE 2

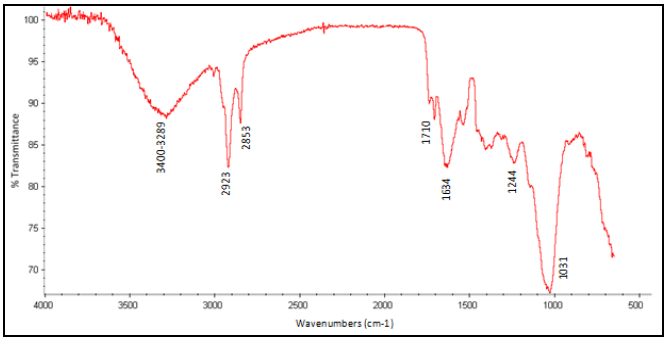

FIG. 24: THE FT-IR SPECTRUM OF *CAPSICUM ANNUUM* POWDER SAMPLE 3

TABLE 10: THE INFERENCE OF FT-IR SPECTRUM OF *CAPSICUM ANNUUM* POWDER SAMPLE 1

Peak's wave length (cm^{-1})	Possible functional group	Compounds may be responsible for peak
3400-3200	N-H and O-H	Ascorbic acid, capsaicin, dihydrocapsaicin, capsorubin, cryptoxanthin and zeaxanthin
2910-2807	C-H	Capsanthin, antheraxanthin, violaxanthin, β- carotene, ascorbic acid, capsaicin
1700	C=O	dihydrocapsaicin, capsorubin, cryptoxanthin, zeaxanthin, thiamine and β-cryptoxanthin. Capsiacin, di hydrocapsaicin, capsanthin and ascorbic acid
1650	C=C	Thiamine
1250-1020	C-O	Capsiacin, di hydrocapsaicin, capsanthin and ascorbic acid

TABLE 11: THE INFERENCE OF FT-IR SPECTRUM OF *CAPSICUM ANNUUM* POWDER SAMPLE 2

Peak's wave length (cm^{-1})	Possible functional group	Compounds may be responsible for peak
3400-3273	N-H and O-H	.Ascorbic acid, capsaicin, dihydrocapsaicin, capsorubin, cryptoxanthin and zeaxanthin
2922-2850	C-H	Capsanthin, antheraxanthin, violaxanthin, β- carotene, ascorbic acid, capsaicin, dihydrocapsaicin, capsorubin, cryptoxanthin
1750	C=O	zeaxanthin, thiamine and β-cryptoxanthin. Capsiacin, di hydrocapsaicin, capsanthin and ascorbic acid
1624	C=C	Thiamine
1238-1031	C-O	Capsiacin, di hydrocapsaicin, capsanthin and ascorbic acid

TABLE 12: THE INFERENCE OF FT-IR SPECTRUM OF *CAPSICUM ANNUUM* POWDER SAMPLE 3

Peak's wave length (cm^{-1})	Possible functional group	Compounds may be responsible for peak
3400-3289	N-H and O-H	Ascorbic acid, capsaicin, dihydrocapsaicin, capsorubin, cryptoxanthin and zeaxanthin
2923-2853	C-H	Capsanthin, antheraxanthin, violaxanthin, β-carotene, ascorbic acid, capsaicin, dihydrocapsaicin, capsorubin, cryptoxanthin
1710	C=O	zeaxanthin, thiamine and β-cryptoxanthin. Capsiacin, di hydrocapsaicin, capsanthin and ascorbic acid
1634	C=C	Thiamine
1244-1031	C-O	Capsiacin, di hydrocapsaicin, capsanthin and ascorbic acid

Scanning Electron Microscopy of Trace Elements: Elemental analysis of test samples were performed on SEM. The SEM image under 3,000 X magnification and the examining area of 1280 X 960 surface were taken for the samples. The surface of the sample 1 is marked with cluster, regular pentagon, triangle touching circle and irregular shape arrangement. The SEM image showed both nano and micro particles sizes within 897 nm to 3.42 µm. These objects showed nanoparticles sizes 897 nm, 897 nm and 948 nm and micro particles sizes 1.29 µm, 1.47 µm, 2.47 µm, 2.98 µm, 3.07 µm and 3.42 µm **Fig. 25**.

While the **Fig. 27** showed the SEM image under 2,000 X magnification and the examining area of 1280 X 960 surface was taken for the sample. The surface of the sample 2 is marked with long ellipse, random shape, equilateral triangle and irregular shape arrangement. These objects showed micro

particles sizes 1.22 µm, 1.70 µm, 1.75 µm, 2.10 µm, 2.33 µm, 3.58 µm and 5.14 µm. While the **Fig. 29** showed the SEM image under 1,000 X magnification and the examining area of 1280 X 960 surface was taken for the sample. The surface of the sample 3 is marked with cluster, torpedo, equilateral triangle and irregular shape arrangement. These objects showed micro particles sizes 2.81 µm, 2.97 µm, 3.06 µm, 3.54 µm, 3.61 µm, 3.93 µm, 3.97 µm, 4.12 µm, 5.82 µm and 6.16 µm.

While the **Fig. 26**, **Fig. 28** and **Fig. 30** showed the SEM-EDAX spectra of trace elements of test samples respectively. While the percentage of trace elements in the samples are shown in **Table 13 - 15** respectively. Generally medicinal plants contain trace elements and they have very important role in body metabolism. Deficiency of trace element in human cause the many diseases [24]. Scanning electron microscope energy dispersive X - ray spectroscopy (SEM - EDAX) is a non-destructive analytical tool to visualize the various nano and micro structures within the sample. It is more useful in the characterization of cluster and crystals [25].

The scanning electron microscope study of samples showed the cluster, regular pentagon, triangle touching circle and irregular arrangement due to the presence of different chemicals and fibrous materials in samples.

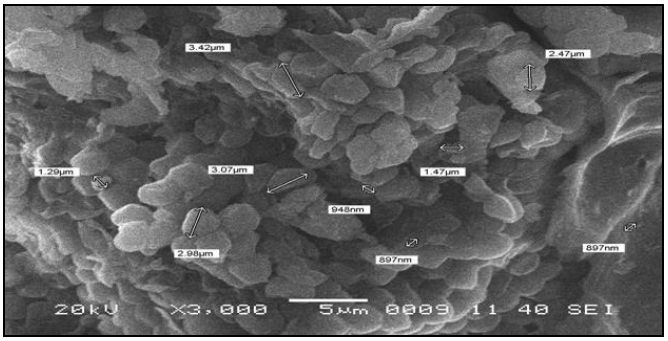

FIG. 25: SEM IMAGE SHOWING NANO AND MICRO PARTICLES IN SAMPLE 1

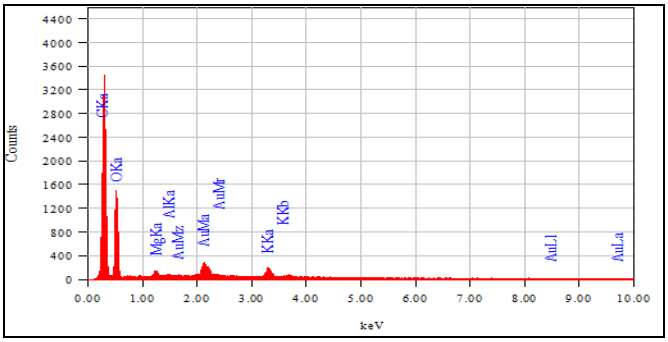

FIG. 26: THE SEM-EDAX SPECTRA OF TRACE ELEMENTS IN SAMPLE 1

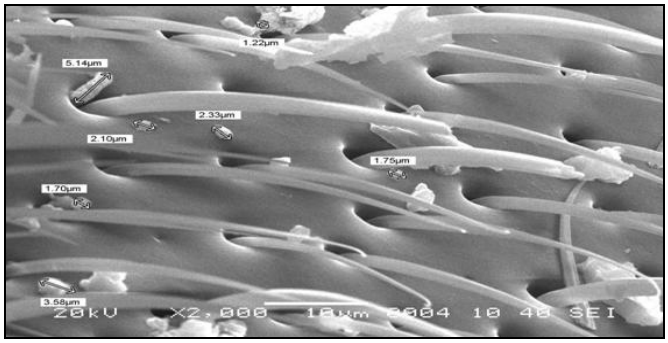

FIG. 27: SEM IMAGE SHOWING MICRO PARTICLES IN SAMPLE 2

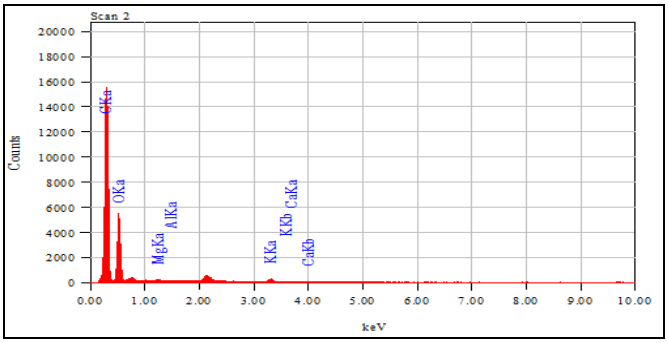

FIG. 28: THE SEM-EDAX SPECTRA OF TRACE ELEMENTS IN SAMPLE 2

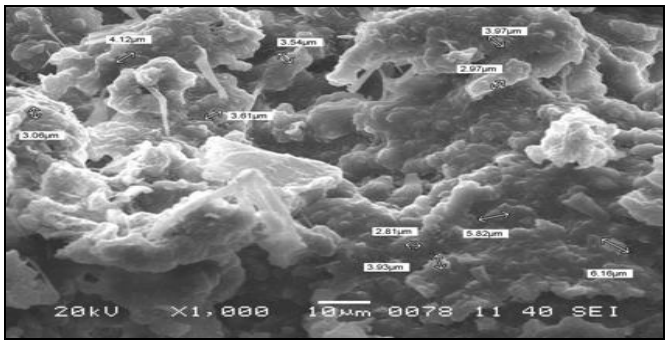

FIG. 29: SEM IMAGE SHOWING MICRO PARTICLES IN SAMPLE

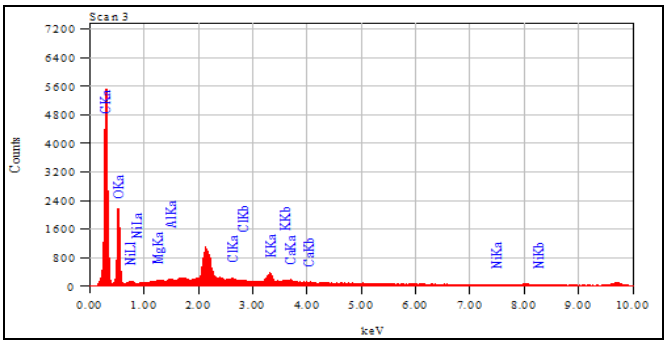

FIG. 30: THE SEM-EDAX SPECTRA OF TRACE ELEMENTS IN SAMPLE 3

The results of trace elements showed that the carbon and oxygen concentration are high in plant they have important role in the function of various enzymes in biological systems, while potassium is needed for muscles contraction [26]. Magnesium needed for regulate of heart rhythm and lowers the cholesterol level, and activate nerves and muscles functions [21]. Calcium prevent the body from blood clotting and help in teeth and bone development [27]. Chloride helpful in food digestion, regulate pH, and with the combination of potassium and sodium maintain electrolyte balance in the body [24, 28]. In all three samples the different trace elements were found with different concentration due to the difference in soil composition in which the spices were grow, or due to the fertilizer, irrigation of water and climate condition [26]. In present study the trace elements found in minimal quantities and heavy metals such as mercury, arsenic, cadmium and lead were not detected in samples in conclusion it can be say that the use of *capsicum annuum* fruit as a medicine is safe.

TABLE 13: PERCENTAGE OF TRACE ELEMENTS IN SAMPLE 1

Element	(keV)	Mass %	Error %	At %
C	0.277	47.75	0.28	57.34
O	0.525	45.94	1.17	41.42
Mg	1.253	0.69	0.49	0.41
Al	1.486	0.11	0.44	0.06
K	3.312	1.23	0.51	0.45
Au	2.121	4.28	1.45	0.31
Total		100.00		100.00

TABLE 14: PERCENTAGE OF TRACE ELEMENTS IN SAMPLE 2

Element	(keV)	Mass %	Error %	At %
C	0.277	52.66	0.25	59.89
O	0.525	46.66	1.31	39.84
Mg	1.253	0.10	0.53	0.05
Al	1.486	0.05	0.47	0.02
K	3.312	0.49	0.54	0.17
Ca	3.690	0.05	0.63	0.02
Total		100.00		100.00

TABLE 15: PERCENTAGE OF TRACE ELEMENTS IN SAMPLE 3

Element	(keV)	Mass %	Error %	At %
C	0.277	50.76	0.25	58.55
O	0.525	46.77	1.22	40.50
Mg	1.253	0.19	0.50	0.11
Al	1.486	0.27	0.44	0.14
Cl	2.621	0.29	0.37	0.11
K	3.312	1.33	0.50	0.47
Ca	3.690	0.32	0.59	0.11
Ni	7.471	0.09	2.19	0.02
Total		100.00		100.00

CONCLUSION: In the present study some distinct data obtained through macroscopic and microscopic features which extended strong information for authentic identification, and purification in preventing from adulteration of red chili fruit from other species. Identification of secondary metabolites such as alkaloids, carbohydrates, saponins, tannins, glycosides and the values of trace elements such as aurum, potassium, magnesium, calcium and chloride determined through SEM-EDAX had further provided confirmation and suitable parameters for the standardization of red chili. The published data are reported to support both academic and industry to utilize the same as reference for further studies and in routine analysis.

ACKNOWLEDGEMENT: The authors are thankful to the entire staff of the Central Science Laboratory, University of Karachi for the technical assistance of SEM-EDAX analysis for the detection of trace elements in test samples.

CONFLICT OF INTEREST: There is no conflict of interest of all authors in this study.

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How to cite this article:
Shaheen N, Imam S, Abidi S, Sultan RA, Azhar I and Mahmood ZA: Comparative pharmacognostic evaluation and standardization of *Capsicum annuum* L. (Red chili). Int J Pharm Sci Res 2018; 9(7): 2807-17. doi: 10.13040/IJPSR.0975-8232.9(7).2807-17.

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