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PHYTOSYNTHESIS OF CADMIUM OXIDES NANOPARTICLES BY CRUDE EXTRACT OF *URTICA DIOICA*

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ABSTRACT

This study was identifying the flavonoids in *Urtica dioica* and using this extract to phytosynthesis of Cadmium oxides nanoparticles: CdO, CdO_2 with characterization about them. The results obtained from the HPLC analysis found nine compounds, which included: Rutin 26.14 % was most abundant, followed by Chlorogenic acid 22.2 %, Naringin, Meyrecetin, Quercetin, Kaempferol, Vanallic acid, Ellagic acid and Isorhamantin.

Two procedures were used to phytosynthesis NPs. The results showed that their average size (calculated by AFM) were: (75.25 and 70.34) nm for procedure1and procedure 2, respectively. From SEM study, there were nanorods particles produced by procedure 1 with dimensions range less than 50 nm, in addition, to nano plate with one diminution lower than 50 nm. While in procedure 2, the particles were spherical nanoparticles crystals with nanorods their sizes were (< 25) nm. The same features were seen in TEM images. The FTIR results showed strong bands are observed at (524.6 and 501.5) cm-1 for procedure 1 and 2, respectively, which attributed to Cd-O stretching vibrations. The XRD patterns of each procedure produce a mixture of nanoparticles: CdO and CdO_2. The average crystallite size of NPs was: (14.74 and 13.97) nm for procedure 1 respectively, and (16.8 and 13.9) nm, respectively. From UV visible spectral, the absorption spectra exhibit strong absorption at 290 nm and 300 nm for procedures (1 and 2), respectively. Their energy band gaps were: (3 and 4.4) eV, respectively.

***KEYWORDS*:** Cadmium oxides; HPLC; Nanoparticles; Phytosynthesis; *Urtica dioica*

1. INTRODUCTION

Urtica dioica L. Urticaceae family (often known as Stinging Nettle) is a herbaceous perennial plant found in temperate and tropical wastelands worldwide (Rani & Bhatia 2021). The hairy leaves and stems carry numerous stinging hairs or trichomes, the ends of which fall off , the hair changes into a needle when touched that will inject many substances such as acetylcholine, histamine, 5-HT (serotonin), moroidin, leukotriens, and potentially formic acid (Asgarpanah and Mohajerani 2012). Flavonoids, tanins, volatile compounds, and sterols are the most frequently recognized phytochemical components from the *U. dioica* (Taheri *et al.*, 2010). Acetylcholine, histamine, and 5-hydroxytryptamine (5-HT) have also been detected in U. dioica. The primary components of U. dioica essential oil are carvacrol (38.2%), carvone (9.0%), naphthalene (8.9%), (E)-anethol (4.7%), hexa-hydro farnesyl acetone (3.0%), (E)-geranyl acetone (2.9%), (E)-ionone (2.8%), and phytol (2.7%). (Majedi *et al*., 2021). Other biologically active substances found in *U. dioica* rhizomes include scopoletin, sterols, fatty acids, polysaccharides, and isolectins (El Haouari & Rosado 2019).

The study and manipulation of materials at length scales between 1 and 100 nm, where special phenomena allow for novel applications, is referred to as nanotechnology. Many different procedures, which may be categorized as bottom-up or top-down, are used to produce nanoparticles.

Nontoxic, ecologically friendly, and biodegradable nanoparticles may be created through biosynthesis (Kuppusamy *et al*., 2014). Biosynthesis to create nanoparticles rather than conventional chemicals for bio reduction and capping (Nande *et al.*, 2021). The biosynthesized nanoparticles have distinct and increased characteristics that make them suitable for biological applications (Hasan, 2015; Koul *et al*., 2021). Many prior studies show that

biosynthesized nanoparticles efficiently decrease oxidative stress, genotoxicity, and apoptosis-related alterations (El-Seedi *et al.,* 2019; Qamar & Ahmad 2021). Furthermore, biological approaches are favoured because they are safe, cost-efficient, simple, and effective sources of high productivity and purity (Salem and Fouda 2021). Many living things, such as bacteria, fungus, yeast, algae, actinomycetes, and plant extracts from different plant parts, such as stem, root, fruit, seed, callus, peel, leaves, and flowers, may create metallic nanoparticles of different sizes and forms (Barabadi *et al.,* 2019; Qamar & Ahmad 2021; Salem & Fouda,2021).A wide variety of metal concentrations and plant extract amounts in the reaction media can change a biosynthesis process, changing the shapes and sizes of the nanoparticles (Shreyash *et al.,* 2021).Cadmium Oxide NPs have recently gained attention from researchers due to their significance, applications, and properties in biomedical applications like anti-bacterial and anti-fungal resistance to bacterial and fungal diseases, as well as optoelectronic devices like solar cells, optical transistors, glassy electrodes, gas sensors, and so forth (Singh *et al.*,2020; Selvi and Sagadevan 2022). CdO nanostructure possesses anti-cancer effects due to its unique physicochemical features (Sagadevan *et al.,* 2021; Shalaby *et al.,* 2022). *Phoenix roebelenii* (Aldeen *et al.,* 2020), tea leaf extracts (Mareedu *et al*., 2021), *agathosma betulina* leaf (Ghotekar, 2019), *Aloe barbadensis miller* extract (Somasundaram *et al*., 2019), and other plant extracts like *Tinospora Cardifolia* (stems), *Rhododendron arboretum* (flower) (Dixit, *et al.,* 2022). Using stinging nettle (*U. dioica L*.) plant extract, Hashem and Salem (2021) developed a green and environmentally acceptable method for producing selenium nanoparticles. They also used this method to create non-regular gold nanoparticles (Molaei *et al.,* 2015). The Debye-Scherrer formula was used to determine the crystallite size of *U. dioica L*. extracts as a green catalyst for the biosynthesis of zinc oxide nanoparticles, which ranged from 19 to 28 nm (Akbarian *et al*., 2018). *U. dioica* leaves extract crystallized in face-centered cubic structure with a size range of 20–30 nm was used in the manufacture of AgNPs (Jyoti, *et al.,* 2016).

The purpose of this work was to identify the flavonoids in the leaves of *U. dioica* and to use this crude extract to phytosynthesize CdO nanoparticles using two different techniques. and analyzing their size, shape, and crystallinity.

2. MATERIALS AND METHODS

2.1. Obtained the plant.

The *Polygonium multiforum radix* and *Urtica dioica* leaves were collected from a local area of Diyala and identified by the Iraqi Ministry of Health's Herbarium using a taxonomic technique. After cleaning and milling, plant leaves were kept in a dark glass bottle in refrigerator.

2.2. *Urtica dioica* crude extract by methanol extraction

A vortex is used to quickly stir one gram of the powdered plant material into glass tubes with 10 mL of a methanol-water solution (9:1) (v/v), after which it is placed in an ultrasonic bath for 30 minutes. The supernatant was then removed from the mixture by centrifuging it for 3 minutes at 4000 rpm. Repeat the foregoing steps using 10 mL of the methanol-water solution (9:1) (v/v) to extract the pellet a second time. In the following step, the two supernatants are mixed and dried using a stream of liquid nitrogen bubbling at 45 °C. The dry residue is recovered using 10 mL of an acetonitrile-water solution (8:2) (v/v), and it is subsequently filtered using HPLC acrodiscs (porosity = 0.45 m).

2.3.Analysis of active ingredient in crude extract equipment

Liquid chromatography 10AV-LC with a binary delivery pump model LC-10A from shimadzu was used for the separation, and a spectrophotometer UV-is 10A-SPD was used to analyze the eluted peaks. Column: was Phenomenex C-18, 3 mm the size of particle was (50 x 2.0 mm I.D) column, mobile phase: 0.05% Trifler acetic acid solvent A; and acetonitrile solvent B. The principal mentioned chemicals were separated under the best conditions. Linear gradients from 0% to 100%. Flow rate 1.0 ml/min. Detection: UV 285 nm.

2.4. The standard materials preparation:

We bought all of our chemicals and standards from Sigma-Aldrich (Steinheim, Germany). In 10 ml of distilled water, 10 mg of each of the following standards materials were dissolved. A decimal dilution series was created using five-fold dilutions. These were the common compounds: (Myricetin, Quercetin, Kaempferol, Vanallic acid, Rutin, Ellagic acid, Isorhamnetin, Chlorogenic acid and Naringin).

2.5. Injection of plant extract in HPLC

The extract sample was diluted to 1/5 in a methanol-acetonitrile combination (v/v), and then fed into the HPLC under the optimal separation conditions. The material was

collected, run through a 0.22 mm disposable filter, and at 4 °C was stored for further analysis. A 20 l sample was added to the HPLC system under the optimal separation parameters indicated above.

CALCULATION:

Concentration of sample (ug/ml) = (Area of sample / Area of standard) * concentration of standard x dilution Factor

2.6. Phytosynthesis of metals NPs by extract of plant.

2.6.1. Preparation of Cadmium oxide bulk particles

Bulk particles of Cadmium oxide (CdO) obtained from book (Chemical Book).Their molecular weight (M.W.) was: (128.413) g/mol. Density 8.15 g/cm3 (crystalline). The average of size was > 3.5 micro meters (> 3500 nm). Purity was: 99.5 %. To make solution of (10 mg/ml) concentrations used distil water.

2.6.2. Phytosynthesis

0.642 g of bulk cadmium oxide particles was added to 1 ml of methanol U. dioica crude plant extract in flask contain 25 ml distal water. On advice hot plate with magnetic stirrer at (60 and 90) Cº for (3 and 9) hours each mixture was placed, respectively. The solution samples, which called colloidal, allowed to cool at room temperature and divided into several sections then kept in screw tubes under freezing. This was repeated three times, (Abdul Jalill, 2018)

2.6.3. Characterizations

The following techniques, including (AFM), Wang and Herron (1991), X-ray diffraction, (Abd, et al., 2020), FTIR, SEM, and TEM analysis (Dong et al., 2015) was used to measure the precise pattern of the fabrication.

3. RESULT AND DISCUSSION

3.1. HPLC analysis

Nine pure compounds was purification, the separation was by column chromatographic (Figure 1). In table (1), showed the analysis of HPLC found that Rutin 26.14 % (929.74) µg/ml was most abundant, followed by Chlorogenic acid 22.2 % (789.34) µg/ml, Naringin 633.87 % (17.82 µg/ml), Meyrecetin 3.28 % (116.52) µg/ml, Quercetin 6.35 % (225.81) µg/ml; Kaempferol 5.589 % (198.73) µg/ml, Vanallic acid 5.67 % (201.62) µg/ml, Ellagic acid 7.145 % (254.25) µg/ml, Isorhamantin 5.83 % (207.15) µg/ml.

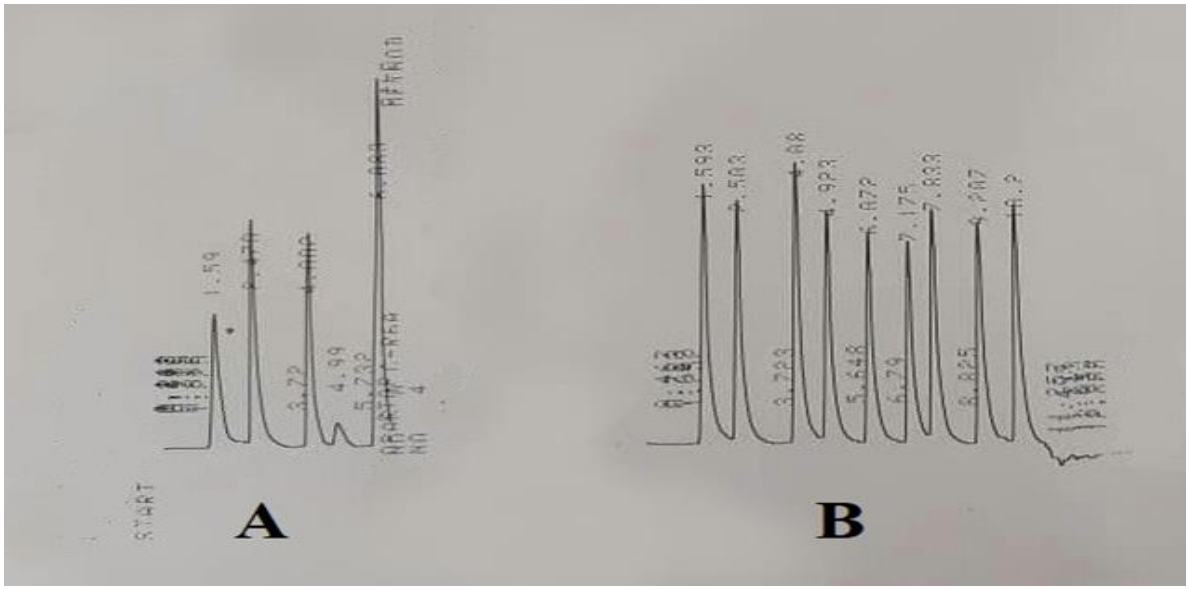


Fig. (1): HPLC Analysis of: (A) *U. dioica* extract, (B) standard compounds.

Table (1): The R.T. and Aru. of reference standard compounds and samples of *U. dioica*

	RSt.	Standard R.T (min.)	Aru. (µvolt)	samples R.T (min.)	Aru. (µvolt)	Con. (µg/ml)	%
1	Myricetin	1.59	162860	1.6	75905	116.52	3.28
2	Quercetin	2.5	164955	2.482	148994	225.81	6.35
3	Kaempferol	4.08	177820	4.138	141351	198.73	5.589
4	Vanallic acid	4.926	160633	4.947	129547	201.62	5.67
5	Rutin	6.072	154345	6.112	574000	929.74	26.14
6	Ellagic acid	7.175	133967	7.188	136240	254.25	7.145
7	Isorhamantin	7.833	180541	7.851	149595	207.15	5.83
8	Chlorogenic acid	9.207	168200	9.187	531066	789.34	22.2
9	Naringin	10.2	184301	10.28	467295	633.87	17.82
							100

RSt. is refer to reference standard; R.T refer to retention time in minuet; Aru. Refer to area under curves (µvolt); Con.refer to concentration

3.2. Phytosynthesis of Cadmium oxide nanoparticles by crude extract of *U. dioica*

3.2.1. AFM analysis :

The average size of procedure 1, which includes heating at 60 Cº for 3 hours) was (75.25 nm), its roughness average (Ra) was 17.4 nm, root mean square (Sq.) was 20.7 nm. Raise the heat of reaction to 90 Cº for 9 hours produce larger nanoparticles. Their average size was 70.34 nm, (Figure 2). Its roughness average (Ra) was 20 nm, root mean square (Sq.) was 23.1 nm. Figure (3) shows AFM topographic images of phytosynthesis nanoparticles.

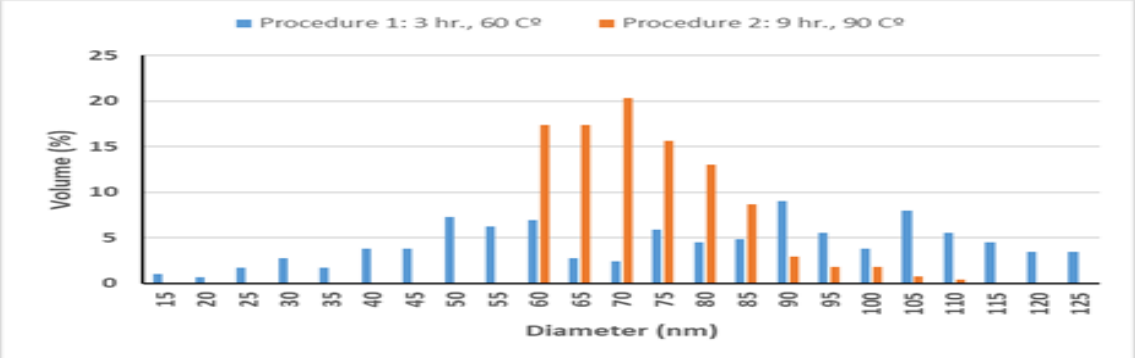
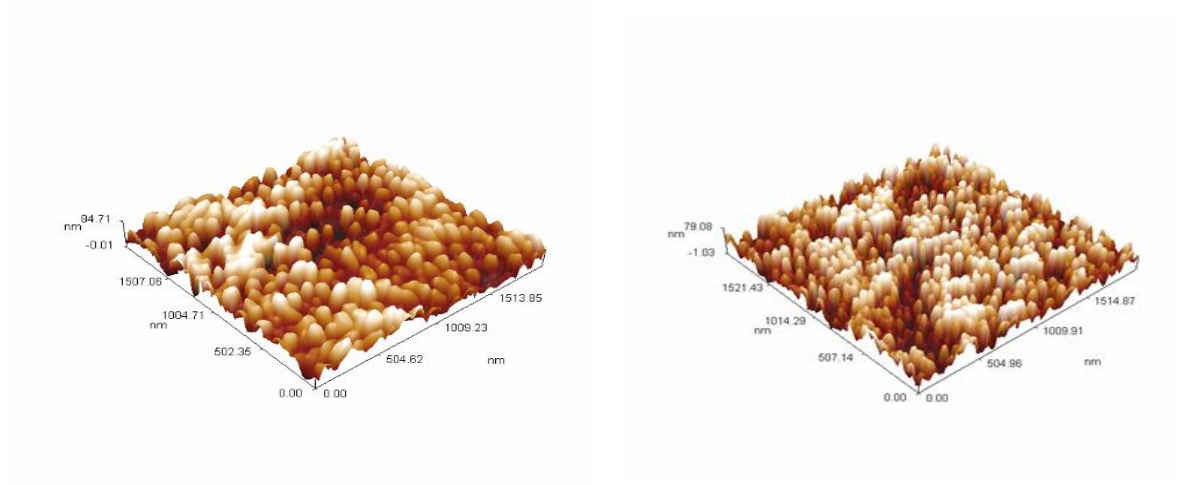


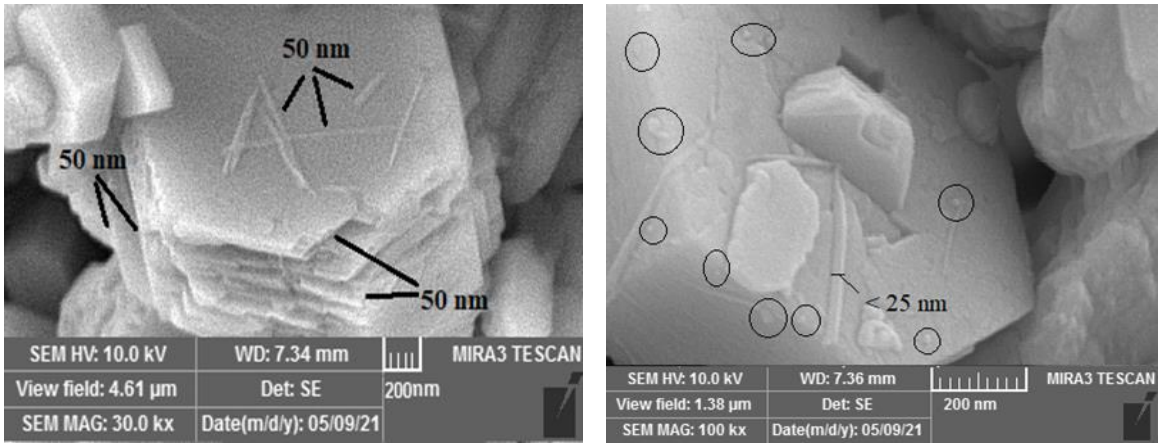
Fig. (2): Granularity volume distribution chart of Cadmium oxide nanoparticles synthesis by procedure (1 and 2) of *U. dioica* crude plant extract



procedure 1 **procedure 2**

Fig. (3): AFM topographic images of Cadmium oxide nanoparticles synthesis by procedure (1 and 2) of *U. dioica* crude plant extract

3.2.2. SEM Analysis: In SEM image of procedure 1 (Figure 4), it can be seen that the particles are nanorods. Each of their dimensions less than (50) nm, in addition to nano plate with one diminution lower than 50 nm. Procedure 2 was used to form spherical nanoparticles crystals with nanorods their sizes were (< 25) nm



procedure 1 **procedure 2**

Fig. (4): SEM image of Phytosynthesis Cadmium oxide nanoparticles using procedure (1 and 2) of *U. dioica* crude plant extract.

3.2.3. TEM analysis:

TEM images of procedure 2 show a nanorods structure less than 50 nm in diameter, which is in accordance with the SEM image presented in (Figure 4) with some non-regular shaped nanoparticles within (100) nm in size.

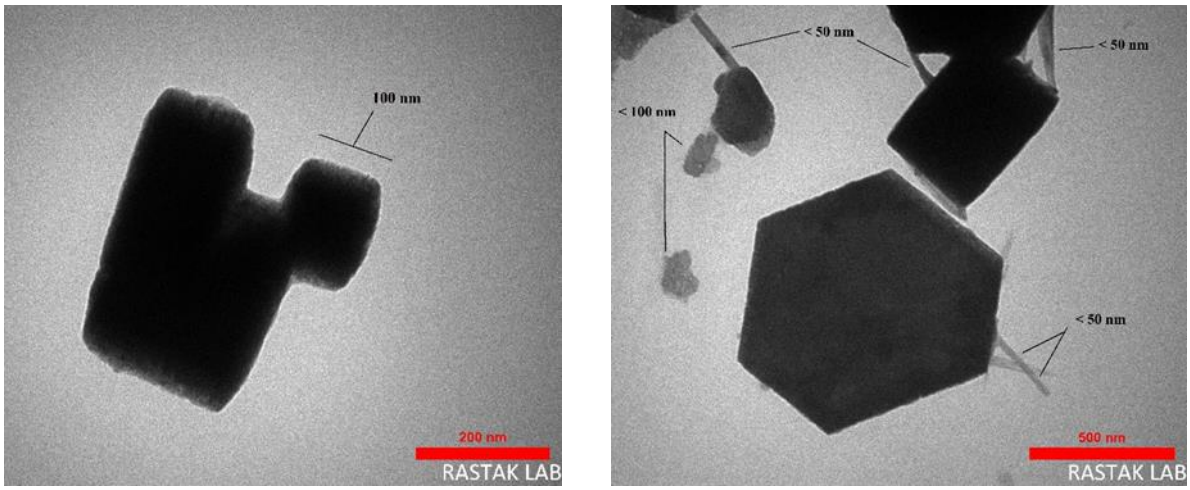


Fig. (5): TEM image of Phytosynthesis Cadmium oxide nanoparticles using procedure 2 of *U. dioica* crude plant extract.

3.2.4. FTIR spectroscopic:

According to Malecka *et al.* (2008), Askarinejad and Morsali (2008), the distinctive bands between 400 and 700 cm-10 belong to the Cd O mode (Mazaheritehrani, *et al*., 2010). Additionally, Cd-O was blamed for the occurrence of weak peak vibrations about 900 - 10 cm (Mahdi, *et al.*, 2019).

Procedure 1: According to Selvam *et al.*, (2020), the band at (3595.3) cm-1, (Figure 5), is ascribed to the hydroxyl stretching vibrations of hydroxyl groups, which are seen in phenols. After Cd (II) biosorption has greatly decreased, the stretching vibration of the hydroxyl group peaks at (3742 and 3672.5) cm-1, indicating that there have been chemical interactions between the metal ions and the hydroxyl groups on the biosorbent surface (Mohammad, *et al.*, 2015). Bands of carboxyl groups for symmetric stretching at 1392.6 cm-1 (Wu *et al.*, 2015). Other organic material was discovered at 1384.9 cm-10 (Figure 5), and C-H bending vibrations (Alkanes) were blamed for it (Pavia, *et al.*, 2014). This suggests significant levels of organic components were present in the cadmium oxide nanoparticles (Al-Hada, 2016). The stretching vibrations of C-O-C, which are both symmetric and asymmetric, are attributed to the band at (1512.2) cm-10 in Figure 5. (Parveen *et al.*, 2014). According to Laskowska *et al.* (2014), the band at 1697 cm-1 is attributable to carbonyl stretching of a hydrogen-bonded acid dimer, while the second one (1739.8 cm-1) is caused by carbonyl stretching of a monocarboxylic acid.

The presence of Cd–O bonds were appeared at: (524.6, 540.1, 597.9, 717.5, and 856.4) cm−1 was confirmed by FTIR spectrum. This is comfortable with other researchers (Balamurugan *et al.*, 2016; Anitha *et al.*,2017; Nasrullah *et al.*, 2020; Abd *et al.*,2020)

Procedure 2: The band at 3595.3 cm-10, (Figure 5) is assigned to the OH stretching vibrations of hydroxyl groups (Selvam *et al.*, 2020). The organic material which found at 1384.9 cm-10 (Figure 5) was attributed to C–H bending vibrations. This indicates that the cadmium oxide nanoparticles were overcome with amounts of the organic components, (Al-Hada, 2016). While the band at 1431.2 cm-10 (Figure 5) is assigned to the asymmetric stretching vibrations of water molecules associated with synthesized CdO NRs (Askarinejad and Morsali, 2008).

The absorption peak at 856.4 cm-10 (Figure 5) corresponds to metal-oxygen stretching of CdO NRs (Kaviyarasu *et al.*, 2014). The formation of fallowing absorption band is attributed to Cd-O stretching vibrations: (501.5, 547.8, and 578.5) cm-10 (Saghatforoush *et al.*, 2012), 678.9 cm-10 (Sivakumar *et al.*, 2015), 714.5 cm-10 (Nasrullah *et al.*, 2020); (847.8, 1384.9) cm-10 (Abd *et al.*, 2020).

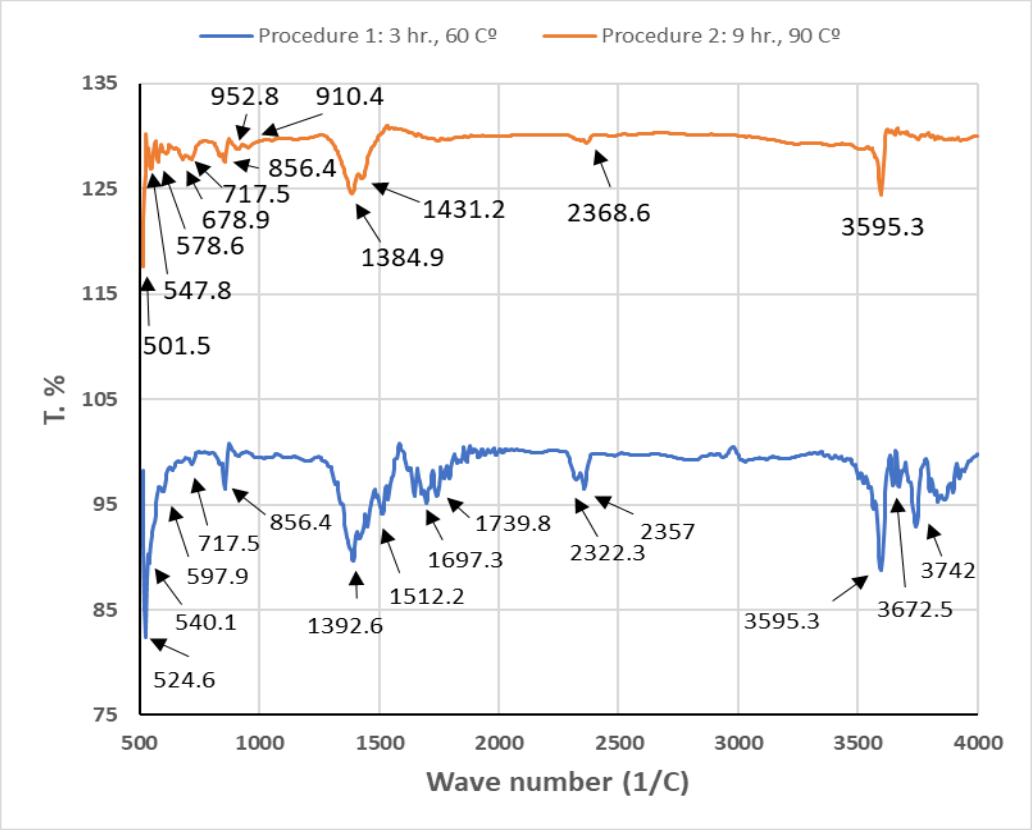


Fig. (6): FT-IR spectra of Phytosynthesis Cadmium oxide nanoparticles using procedure (1 and 2) of *U. dioica* crude plant extract.

3.2.5.X-ray diffraction:

Each of procedures 1 and 2 (which includes heating at 60 Cº and 90 Cº for 3 hrs. and 9 hrs. respectively) produced mixture of nanoparticles: CdO and CdO_2. Their 2 theta, planes, strain value, dislocation density, average crystallite size are illustrated in table (2). The results showed that NPs were cubic crystals matched with Cards No. which found in front of each theta in table (2).

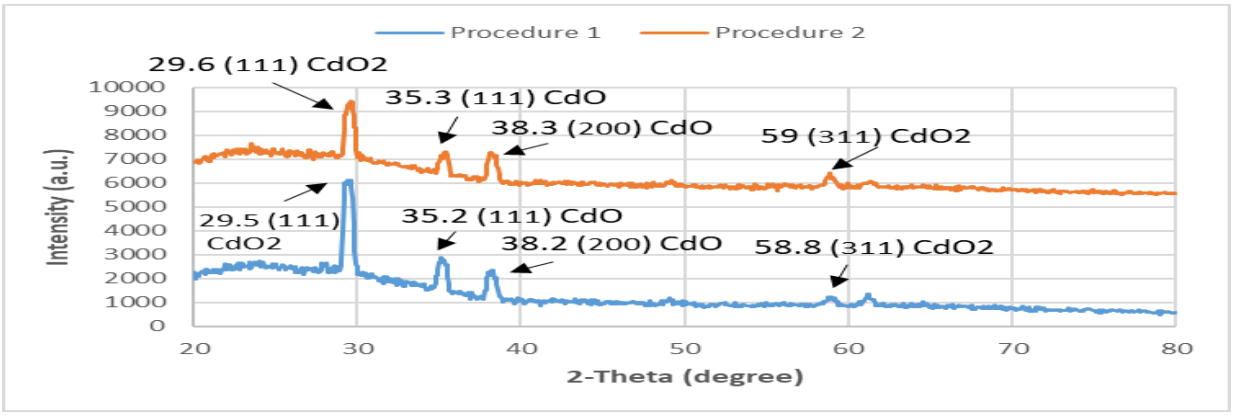


Fig. (7): X-ray pattern of Phytosynthesis Cadmium oxide nanoparticles using procedure (1 and 2) of *U. dioica* crude plant extract.

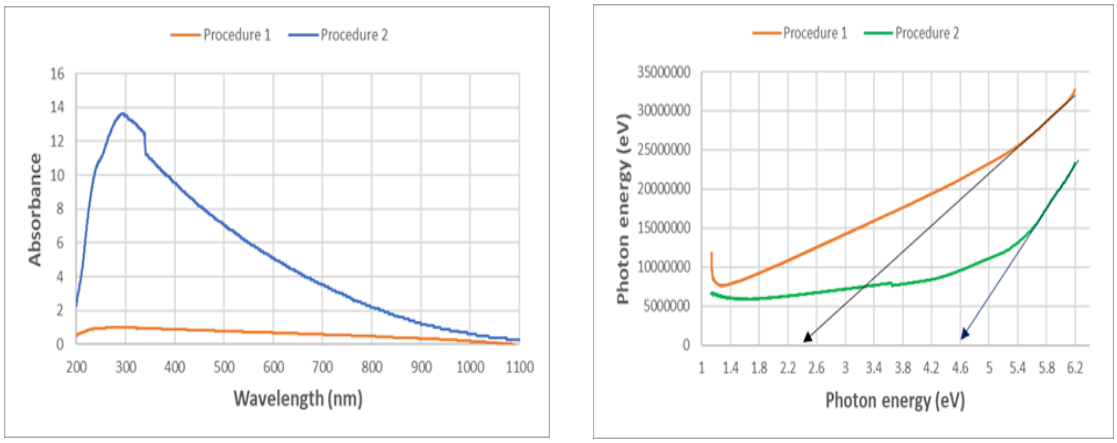
Table (2): Summary of X-ray characterization of Phytosynthesis Cadmium oxide nanoparticles using procedure (1 and 2) of U. dioica crude plant extract.

Sample	Planes (hkℓ)	2 theta (DEG)	FWHM (DEG)	D (nm)	STRAIN XE-4	DIS X1014	Card No.	Matching with other ref.	Matching with other ref.
Procedure 1	111 (CdO2)	29.7	0.75	10.90	127.14	84.15	(JCPDS file No. 039-1221:CdO2)	(Skheel *et al.*, 2021)	(Skheel et al., 2021)
	(100)Cub. CdO	35.2	0.65	12.76	108.64	61.44	96-900-6691	(Naser *et al*., 2019)	(Naser et al., 2019)
	(200) CdO	38.15	0.50	16.73	82.85	35.73	(JCPDS card No. 65-2908)	(Yufanyi *et al.*,2014)	(Yufanyi et al., 2014)
Ava. CdO	311 CdO2	58.8	0.65	13.97	99.2	51.2	(JCPDS 005-0640)	(Moreno *et al*.,2011)	(Moreno et al 2011)
Ava. CdO2				14.74					
				13.97					
Procedure 2	111 (CdO2)	29.55	0.80	10.22	135.66	95.81	(JCPDS file No. 039-1221 :CdO2)	(Skheel *et al.*, 2021)	(Skheel et al., 2021)
	(200) Cub CdO.	35.6	0.55	15.09	91.82	43.89	CdO 96-900-6691	(Naser *et al.*, 2019)	(Naser et al., 2019)
	(200) CdO	38.3	0.45	18.60	74.53	28.92	(JCPDS card No.65-2908)	(Yufanyi *et al.*, 2014)	(Yufanyi et al., 2014)
Ava. CdO	311 CdO2	59	0.65	13.99	99.10	51.12	(JCPDS 005-0640	(Moreno *et al*.,2011)	(Moreno et al 2011)
Ava. CdO2				16.8					
				13.9					

(hkℓ) planes: crystallographic plane; FWHM: Full width at half maximum; D: dimension of Crystal in nm; η x10^{-4}: strain value; δ x 10^{14}: dislocation density; Ava.: Average.

3.2.6. UV–visible analysis:

UV–visible spectral results of procedures 1 and 2 (which includes heating at (60 and 90) C° for (3 and 9) hours showed up that the absorption spectra exhibit strong absorption at 290 nm and 300 nm, respectively. Their energy band gaps were:(3 eV and 4.4 eV),(Figure 7:A and B).



(A) (B)

Fig. (8): (A) absorption spectrum and (B) (ahv) 2 versus photon energy of Phytosynthesis Cadmium oxide nanoparticles using procedure (1 and 2) of U. dioica crude plant extract.

In view of identifying the mechanism of the synthesis of the CdO NPs, the bioactive compounds in U. dioica are identified. The column chromatographic separated and purified nine pure compounds.

Currently, two procedures were success to produce CdO in nano size. FT-IR analysis of phytosynthetic CdO NPs found some bands attributed to the phenols, Alkanes in addition to some organic components. These could be responsible for the reduction of Cd++ ions and formation of the corresponding CdO NPs (Al-Hada, 2016). Choudhary and his team synthesized CdO NPs by using fruit peel of Malus domestica which confirms the bonding of phenols to metal species (Choudhary et al., 2020). In addition, the ability of a plant extract to synthesis NPs might due to the presence of dextrose in it, this is supported by what Sadhukhan and his team mentioned in their paper, (Sadhukhan et al., 2019).

In the current investigation, the energy band gaps of the NPs were: (3 eV and 4.4 eV). It was greater than the typical band gap of 2.2–2.5 eV reported by Thohogi et al. (2016) and Mohanraj et al. (2017). (Kondawar et al., 2011). Through the quantum size effect, it might rise from 2.5 eV to 5.8 eV from CdO bulk to CdO thin film (Abd et al., 2018). However, it is noted that the quantum size effect causes the direct band gap energy of green-synthesized Cd NPs to be somewhat greater than that of Cd nanocomposite (3.09 eV) and bulk CdO (2.5 eV).

4. CONCLUSIONS

Five flavonoids are present in the leaf extract of *U. dioica*. CdO nanoparticles were produced using two different techniques. The findings show that all processes for producing CdO in nano size were successful. It will be beneficial to investigate how certain active *U. dioica* chemicals contribute to the phytosynthesis of CdO nanoparticles.

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الخلاصة

هدفت الدراسة الحالية الى الكشف عن مركبات الفلافونويدات الموجودة في نبات القريص واستخدام المستخلص الخام لهذا النبات في التخليق النباتي لجسيمات أكاسيد الكادميوم النانوية: CdO وCdO2وتشخيصها . اظهرت نتائج تحليل كروماتوغرافيا السائل عالية الأداء HPLC وجود تسعة مركبات في النبات ، شملت: Rutin 24.14% وهو المركب الأكثر وفرة ، يليه Chlorogenic acid 22.2%, Naringin ، Myricetin ، Quercetin ، Kaempferol ، Vanallic acid ، Ellagic acid و Isorhamantin. تم استخدام طريقتين (1 و 2) للتخليق النباتي للمواد النانوية، أظهرت النتائج أن متوسط حجم الجسيمة النانوية (محسوبًا بواسطة مجهر القوة الذرية): (75.25و 70.34) نانومتر باستخدام الطريقتين (1 و 2) على التوالي. وأظهرت نتائج دراسة المجهر الالكتروني الماسح SEM كانت الجسيمات بشكل قضبان نانوية وباستخدام الطريقة (1) وكانت أبعادها أقل من 50 نانومتر ، بالإضافة إلى انتاج صفائح نانوية ذات بعد واحد أقل من 50 نانومتر. بينما عند إستخدام الطريقة (2) ، كانت الجسيمات عبارة عن بلورات كروية وقضبان نانوية كان حجمها (< 25) نانومتر. نفس هذه الصفات شوهدت في صور المجهر الالكتروني النافذ TEM. اظهرت نتائج تحليل مطياف الاشعة تحت الحمراء FTI R وجود اواصر قوية عند (524.6 و 501.5) سم -1 عند إستخدام الطريقتين 1 و 2 على التوالي ، وهي تُعزى إلى Cd- O. أظهرت نتائج حيود الاشعة السينية XRD لكل طريقة مستخدمة خليطًا من الجسيمات النانوية: CdO و CdO_2. كان متوسط حجم البلورات النانوية (14.74 و 13.97) نانومتر للطريقة (1) على التوالي ، و (16.8 و 13.9) نانومتر ،للطريقة (2) على التوالي . أظهرت أطياف الامتصاص (باستخدام فحص مطياف الاشعة فوق البنفسجية) امتصاصًا قويًا عند 290 نانومتر و 300 نانومتر للطريقتين (1 و 2) على التوالي. كان نطاق فجوات الطاقة: (3 و 4.4) فولت ، على التوالي.

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