# PHYTO-SYNTHESIS OF CdO NPs BY CRUDE EXTRACT OF *POLYGONUM MULTIFORUM*

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**ABSTRACT**
**The goal of this research is to detect flavonoids in Polygonum multiforum and use these extracts in the phyto-synthesis of (CdO NPs) Nano-particles (CdO, CdO2) and characterize them. The HPLC examination yielded five compounds: Emodin (29.55%), followed by Aloe-emodin (24.52%), Chrysophanol (19.24%), Rhein (16.61%), and Physcion (10.06%). Two techniques were utilized to synthesize NPs, and the average size (by AFM) was (83.26 and 86.14) nm for the first and second procedures, respectively. SEM analysis revealed that process 1 produced Nano-rod particles with diameters ranging from (20-50) nm, whereas procedure 2 produced hexagonal Nano-plate crystals with a single Nano-scale size (20-100) nm. TEM pictures showed the same characteristics. For processes 1 and 2, the FTIR analysis showed prominent bands at 524 and 532.4 (1/cm), respectively. This was ascribed to stretching vibrations in Cd-O.**

**Keywords: CdONano-particles; flavonoids; phyto-synthesis; HPLC; Emodin.**



"ألتوليف النباتي لجسيمات أكاسيد الكادميوم النانوية عن طريق المستخلص الخام من نبات عصا الراعي"

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**المستخلص**

**هدفت الدراسة الى الكشف عن مركبات الفلافونويدات الموجودة في نبات عصا الراعي واستخدام المستخلص الخام لهذا النبات في التوليف النباتي لجسيمات أكاسيد الكادميوم النانوية:CdO،CdO2 وتشخيصها. اظهرت نتائج تحليل كروماتوغرافيا السائل عالية الفعالية HPLC وجود خمسة مركبات في النبات، شملت: مركب Emodin وهو المركب الاكثر وفرة (29.55 %), يليه Aloe-emodin (24.52%)Chrysophanol,(19.24%) Rhein,(16.61%) وPhyscion (10.06%). تم استخدام طريقتين (1 و 2 للتوليف النباتي للمواد النانوية، اشارت الدراسة الى أن معدل حجم الجسيمات النانوية محسوبًا باستخدام "مجهر القوة الذرية" كانت: (83.26 و86.14) نانومتر باستخدام الطريقتين (1 و 2) على التوالي كما اشارت دراسة "المجهر الالكتروني الماسح" SEM ان هذه الجسيمات بشكل "قضبان نانوية" وباستخدام الطريقة (1) وكانت أبعادها تتراوح بين (20–50) نانومتر، بالإضافة إلى انتاج صفائح نانوية ذات بعد واحد أقل من 50 نانومتر. بينما عند إستخدام الطريقة (2)، كانت الجسيمات عبارة عن صفائح نانوية بلورية سداسية ذات بعد واحد يتراوح بين (20–100) نانومتر. شوهدت نفس هذه الصفات في صور المجهر الالكتروني النافذ TEM. اظهرت النتائج في تحليل مطياف الاشعة تحت الحمراء FTIR وجود اواصر قوية عند (524 و 532.4) سم −1 عند إستخدام الطريقتين 1 و 2 على التوالي. وهو ما يعزى إلى قوى اهتزازات Cd-O.**

**الكلمات المفتاحية: اكسيد الكادميوم , فلافونويدات، التوليف النباتي، كروماتوغرافيا السائل عالية الاداء , ايمودين.**

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## INTRODUCTION

Nanoparticles (NPs), a broad family of materials with diameters under 100 nm, are used in a variety of sectors, Due to their diverse characteristics, these include medicine, pharmacology, manufacturing and materials, the environment, electronics, energy collecting, and mechanical (20, 38). (CdO NPs), which have explicit and implicit energy gaps for the band equal to 2.31 eV and 1.360 electron volt, respectively, have been found to be advantageous in photo-catalytic applications such as solar cells, photo-voltaics, flat displays, and sensors (42). (CdO NPs) Nano-structures are a low-cost(39), chemically stable, and comparatively nontoxic metal oxide semiconductor Nano-materials with applications in photo- catalysis (19),gas sensors, transistors, transparent electrode, and solar cells, drug delivery, antibacterial medicines, optics, and environmental cleanup (12). The exceptional characteristics of CdO nanoparticles are caused by the large number of surface atoms they contain. Even though there are physical and chemical methods for creating nanoparticles, there are drawbacks to using them, such as hazardous products, excessive energy consumption, and the use of toxic solvents. (34). CdO NPs have a gap energy of roughly 4.05 eV and a greater exciton binding energy of 75 meV when compared to other semiconductors (18). Several chemical methods have been used to create the nano-structure of CdO NPs (3,33). The majority of techniques are rather costly and intricate, particularly when it comes to regulating particle size and homogeneity (15). .The use of plant extracts to synthesize Nano-particles is known as phyto-synthesis. This technique may be more favorable than microorganisms since it is easier to scale up biohazards and requires a more complex procedure for sustaining cell cultures (30).

Because plant extracts include specific phytochemicals, they have been utilized in a number of studies to promote the reduction of metal nanoparticles (2, 11, 14, 17, 21). Flavonoids are the primary phytochemicals responsible for the sharp reduction; to far, more than 4000 flavonoids derived from plants have been found. Green plants are more likely to contain them, mostly as glycosides in the “leaves, flowers, stems, and roots. Chalcones, flavones, flavanols, flavanones, anthocyanins, and isoflavones” are the main flavonoids that are frequently brilliantly colored (40), as well as tannins, cardiac glycosides, and saponins. They also bind to proteins as capping on nanoparticles (27). Flavonoids are composed of two benzene rings. phenolic compounds, and flavonoids (4).NPs, also, may be synthesized by using terpenoids, alkaloids and some flavonoids as a reducing agent (13, 22), quercetin (16), Kaempferol (10), hesperidin, naringin and diosmin (29). The production of (CdO NPs) Nano-particles using phytosynthesis lowers the toxicity of the material and makes it environmentally benign. The Queen of herbs, "Ocimum Sanctum" (holy basil), was used for the first time to create this environmentally friendly biosynthesis of CdO NPs (1). Emodin 3-methyl-1,6,8-trihy-droxy-anthraquinone is the most common natural anthraquinone, found in plants, lichens, and fungus. Plants in the "Poly-gonaceae family, such as Poly-gonum multiflorum, Rheum palmatum, tanguticum, officinale, and Poly-gonum cuspidatum”, are common sources of emodin. The structure of emodin, which contains many hydroxyl and carbonyl groups that can chelate with metal ions in bio-logical target enzymes to form rather stable chelates, is one of the primary factors contributing to its wide range of biological action (44).

## MATERIAIS AND TECHNIQUES

**Obtained the plant**: *Polygonum multiforum*/ family Polygonaceae was brought from the local market and actualized by "the Herbarium of Iraqi Ministry of Health according to a taxonomic procedure. After cleaning and milling, plant leaves were kept in a tenebrous glass bottles in fridge".

**Methanol extraction of *polygonum multiforum* crude extract:** Ten milliliters of a methanol-water solution (9.1) (v/v) are added to glass tubes containing one gram of powdered plant leaves. The mixture is aggressively swirled with a vortex before being submerged in an ultrasonic bath for half an hour. After centrifuging the mixture for 3 min. at 4000 rpm, the supernatant was extracted. Using 10 mL of methanol-water solution (9.1) (v/v), the pellet is extracted once more and goes through the identical procedures as before. The combination of the

two supernatants is then evaporated to dryness using a stream of liquid nitrogen bubbling at temperature 45 °C. The dry residue is recovered with 10 mL of a water-acetonitrile mixture (8.2) (v/v) then filtered through HPLC acrodiscs (porosity = 0.45 µm).

**Equipment for analyzing the active component in crude extract:** The split took place. A UV-Vis 10 A- SPD spectro-photometer was used to analyze the eluted peaks using liquid chromatography, and a "Shimadzu 10AV-LC with a binary delivery pump model LC-10A Shimadzu was used. Under ideal circumstances", the primary compounds were separated. Column: Phenomenex C-18, 50 x 2.0 mm I.D., 3 µm particle size. Mobile phase: linear gradients from 0% to 100% using 0.05% Trifler acetic acid solvent A and acetonitrile solvent B. Rate of flow: 1.0 mL/min. The wavelength at which UV is detected is 285 nm.

**Preparation of standard compounds:** All Sigma-Aldrich (Steinheim, Germany) provided the chemicals and standards. 10 mL of distilled water should be used to dilute 10 mg of each of the following standard compounds. Fivefold dilutions were combined to generate a decuple dilution series. . The typical chemicals included "myrecetin, quercetin, kaempferol, vanallic acid, rutin, ellagic acid, isorhamantin, chlorogenic acid, and naringin".

**Injection of plant extract in HPLC:** The extract sample was injected into the HPLC under ideal separation conditions after being diluted to one-fifth in a methanol-acetonitrile mixture (v/v). After passing through a disposable 0.22 µm filter, the extracted material was stored at 4°C for further examination. Utilizing the ideal separation conditions mentioned above, inject 20 µl of the sample into the HPLC system (37).

**Calculation:**

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

**Phyto-synthesis of metals NPs by extract of plant:**

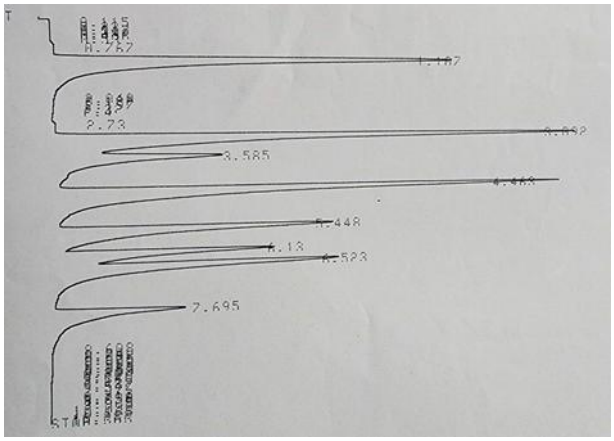
**1. Preparation of (CdO NPs) bulk particles:** Bulk particles of (CdO NPs) obtained from Chemical Book. Their molecular weight was: (128.413) g/mol. Density 8.15 g/cm3 (crystalline). Their rate of size was > 3.5 micro meter (> 3500 nm). Purity: 99.5 %. Distil water was utilized to make solution of (10 mg/ml) concentrations.

**2. Phyto-synthesis:** In a flask containing only 25 mL from cold water, 0.642 g of bulk (CdO NPs) particles were combined with 1 mL of methanol "P. multiforum" extract of crude plant . Every material mixture was heated for three to nine hours at 60 to 90 degrees Celsius on a magnetic stirrer hot plate. Colloidal solution samples were cooled to room temperature, separated, and then frozen in screw tubes. Three iterations of the procedure were conducted (2).All HPLC steps were performed at "Al-Rawabi Al-Khadhra Company for Chemical and Environmental Studies and Analysis, Ltd." in Baghdad, Iraq".

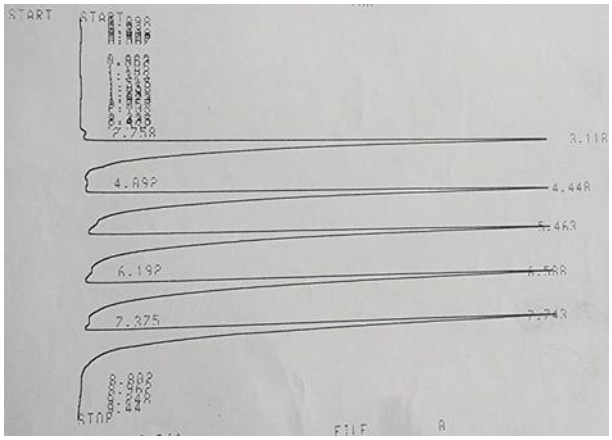
**3. Characterizations:** Using the fallowing procedures, the precise pattern of the manufactured crystals' morphology, average structure, phase purity, particle size, and distribution are described and measured. (AFM), SEM , TEM analysis, and X-ray diffraction (7,24,26).

**RESULTS AND DISCUSSION**

**HPLC analysis:** The column chromatographic separations resulted in the isolation of five pure chemicals (Figure 1). The HPLC study revealed that Emodin (29.55%) (217.34 µg/ml) was the most abundant, followed by Aloe -emodin (24.52%) (180.36 µg/ml), Chrysophanol (19.24%) (141.56 µg/ml), Rhein (16.61%) (122.21 µg/ml), and Physcion (10.06%) (74 µg/ml).




**A**



B

**Fig . 1 . HPLC Analysis of: (A) standard compounds, (B) "P. multiforum "extract.**

**Phyto-synthesis of (CdO NPs) Nano-particles by crude extract of *P. multiforum:*** AFM: Procedure 1, which involves heating at 60 degrees Celsius for three hours, had average diameters of 83.26 nm, a roughness average (Ra) of 10.1 nm, and a root mean square (Sq.) of 11.9 nm. Larger Nano-particles were produced by raising the heat of reaction to 90 Cº for nine hours; on average, they measured 86.14 nm (Figure 2). Its root mean square (Sq.) was 6.68 nm, while its roughness average (Ra) was 5.88 nm. AFM topography pictures of phytosynthesis Nano-particles were displayed in Figure (3).

**Table 1.The retention time (RT) and area under curves of reference standard compounds and samples of P. multiforum**

| No. | RSt. | Standard | | Samples | | | |
|---|---|---|---|---|---|---|---|
| | | R.T. (min) | Aru.(µvolt) | R.T(min.) | Aru.(µvolt) | Con. (µg/ml) | % |
| 1 | Aloe - emodin | 3.11 | 291858 | 3.092 | 210567 | 180.36 | 24.52 |
| 2 | Emodin | 4.4 | 237889 | 4.463 | 206797 | 217.34 | 29.55 |
| 3 | Rhein | 5.46 | 230033 | 5.118 | 112456 | 122.21 | 16.61 |
| 4 | Chrysophanol | 6.5 | 218747 | 6.523 | 123869 | 141.56 | 19.24 |
| 5 | Physcion | 7.74 | 223278 | 7.695 | 66093 | 74 | 10.06 |
| | | | | | | | 100 |

**RSt.: reference standard; R.T: retention time in minuet; Aru.: area under curves (µvolt); Con.; concentration**

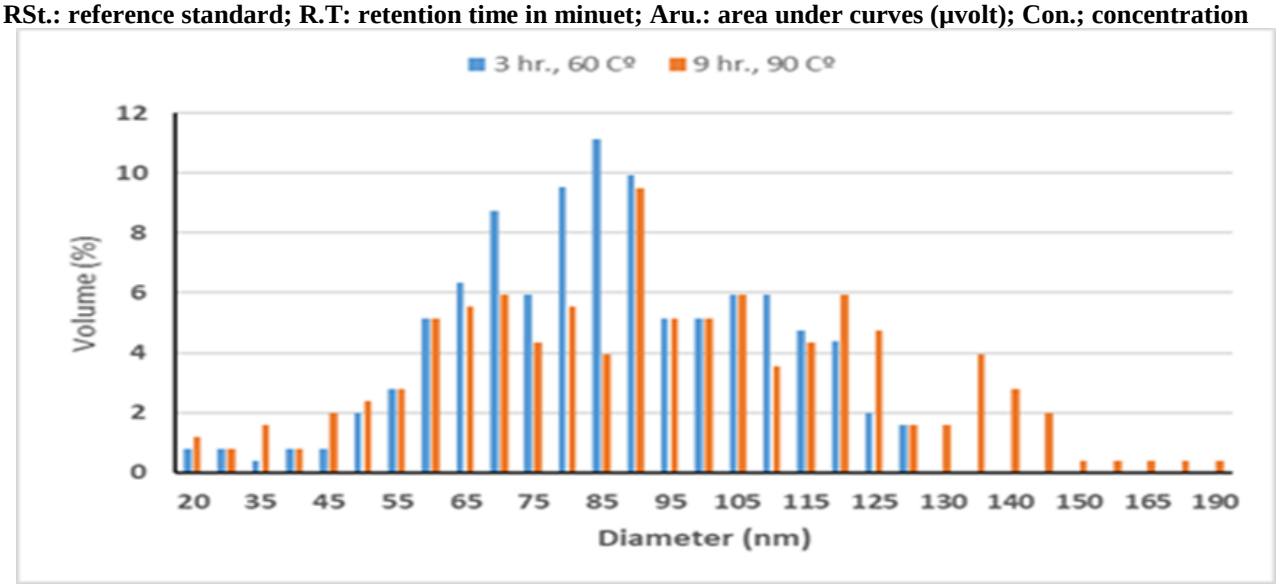
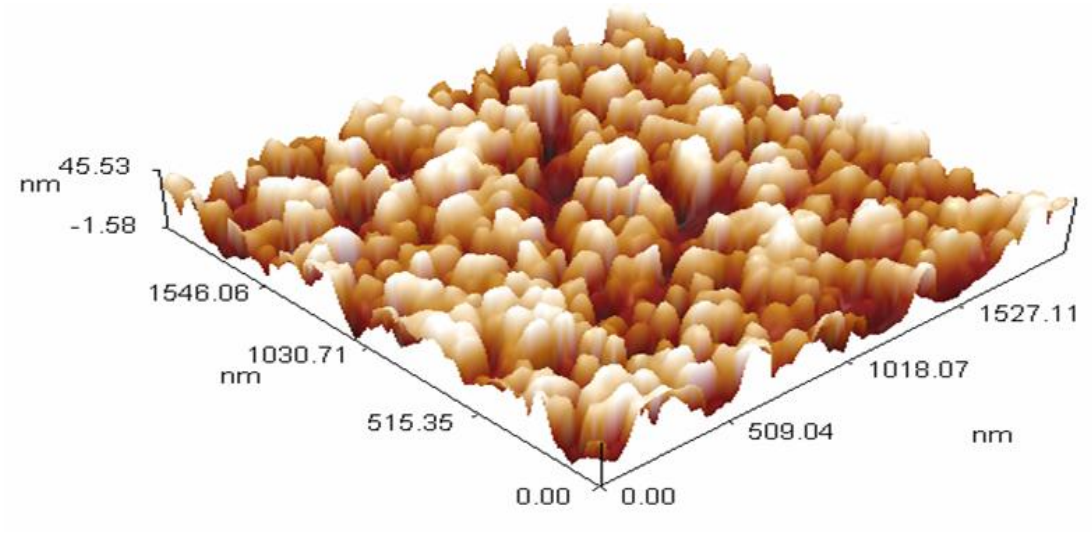
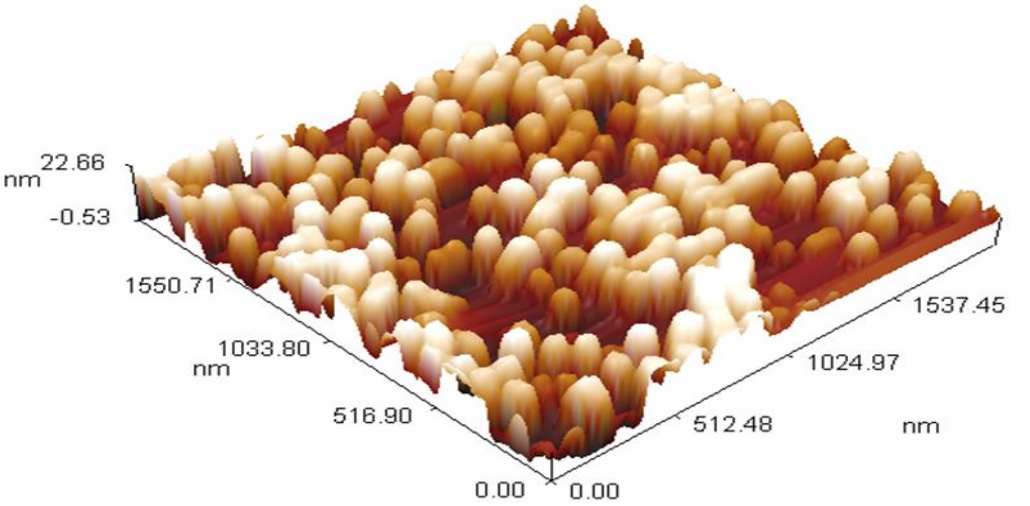



**Fig. 2. distribution chart of (CdO NPs) Nano-particles synthesis by procedure (1 and 2) of *"P. multiforum* "extract of crude plant**



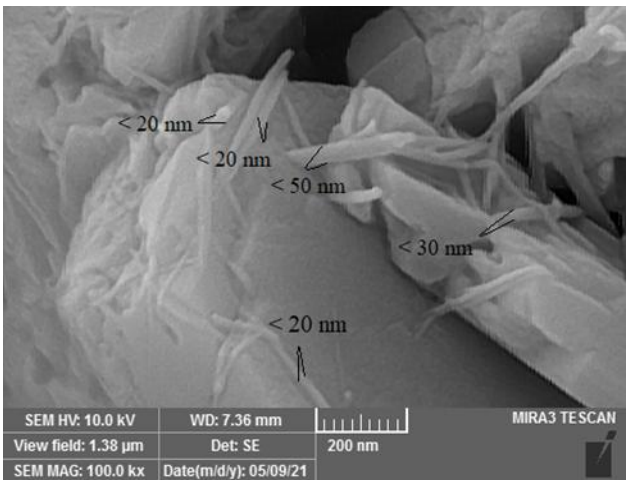

**(A) Procedure1**



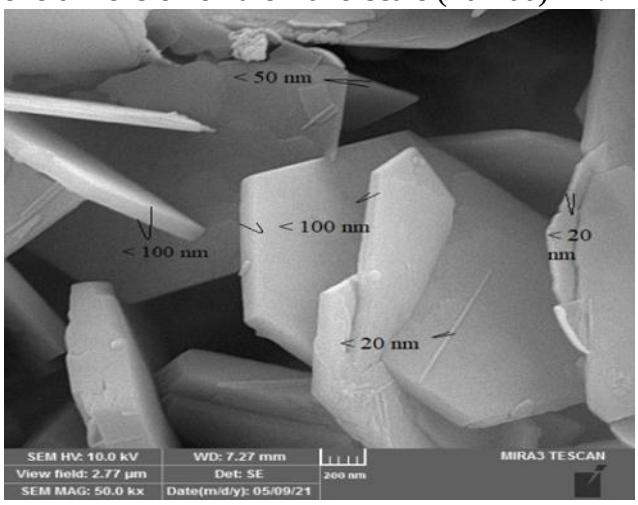

**(B) Procedure2**

**Fig. 3. AFM topographic images of (CdO NPs) Nano-particles synthesis by procedure (1 and 2) of *"P. multiform* "extract of crude plant**

**SEM Analysis**: In SEM image of procedure 1 (Figure 4), it can be seen that the particles are Nano-rods. Each of their dimensions range from (20-50) nm. Procedure 2 was using to form hexagonal Nano-plate crystals having one dimension on the Nano-scale (20-100) nm.




**(A) Procedure 1.**




**(B) Procedure 2**

**Fig. 4. SEM image of phyto-synthesis (CdO NPs) Nano-particles using procedure (1 and 2) of *"P. multiforum* "extract of crude plant**

**FTIR spectroscopic** The bands in Procedure 1 were comparable to those in Procedure 2. The band at 3599.2 (1/cm)(Figure 5) corresponds to the OH stretching vibrations of hydroxyl groups (32). The organic substance discovered at 1384.9 (1/cm)(Figure 5) was due to "C-H bending vibrations". This shows that the (CdO NPs) Nano-particles were overcome by the organic components(8). While the band at 1431 (1/cm)(Figure 5) corresponds to the asymmetric stretching vibrations of water molecules associated with manufactured CdO NRs(9). The absorption peak at 856 (1/cm) as shown in (Figure 5) which corresponds to metal-oxygen stretching of CdO NPs(23). The formation of absorption band 524 (1/cm)and 532.4 for procedure 1 and 2, respectively, due to Cd-O stretching vibrations(28). It has been found that the intense IR bands around 500 and 600 are the characteristic bands of CdO those similar with (5,9,25).

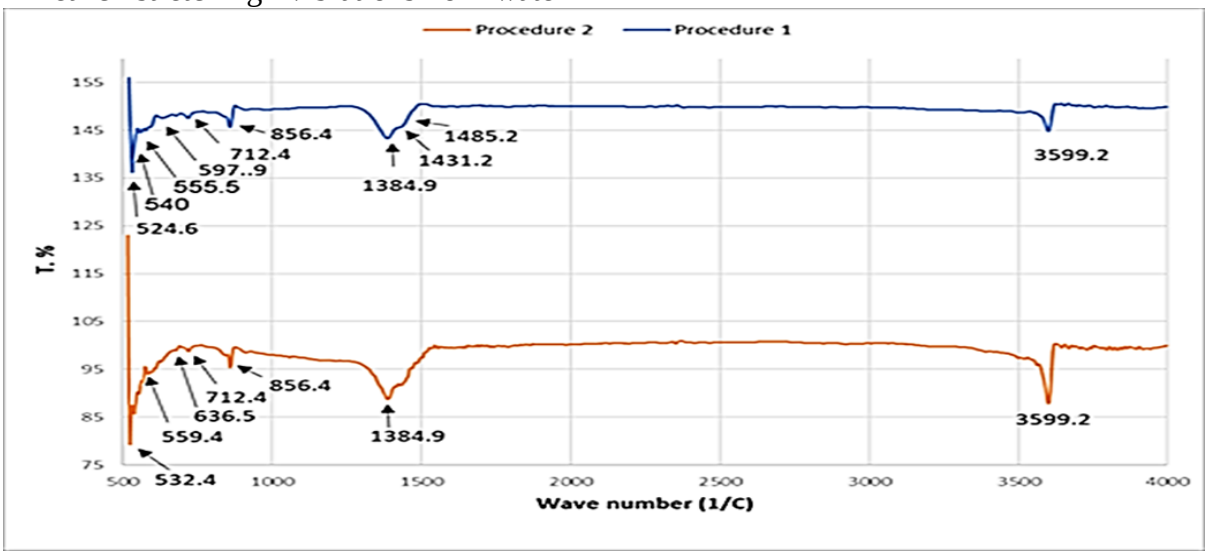



**Fig. 5. FT-IR spectra of Phyto-synthesis (CdO NPs) Nano-particles using procedure (1 and 2) of *"P. multiforum* "extract of crude plant**

**X-ray diffraction:** Procedures 1 and 2 create a combination of Nano-particles (CdO and CdO2) after heating at 60 and 90°C for 3 and 9 hours, respectively. The CdO planes with h k l values were 32.3 (111) and 38.1 (200), respectively (Figure 6). Matching with cubic CdO (JCPDS 05-0640) suggested the synthesis of this compound. According to Scherer's equation (6), their average nano--particle crystallite sizes were 11.95 nm and 13.95 nm, respectively. Table (2) displayed their dislocation density and strain value. 29.4 (111) and 58.8 (311) were the CdO2 planes with h k l values; these results are comparable to those of (31,35,36,41). *All analyses pertaining to Nano-- were conducted at Photo-n Center Baghdad. Iraq

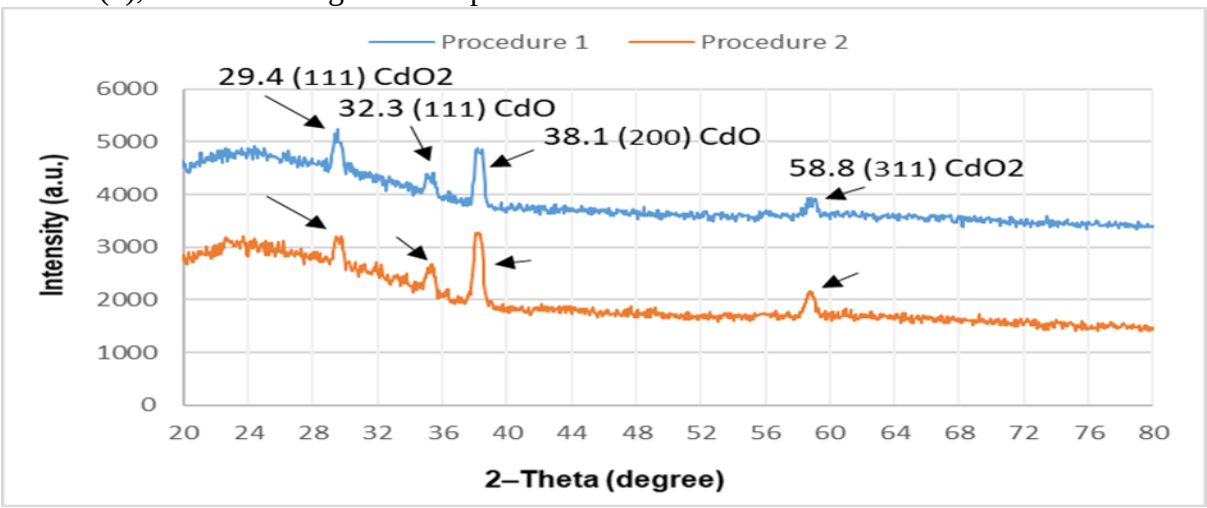



**Fig.6. X-ray pattern of Phyto-synthesis (CdO NPs) Nano-particles using procedure (1 and 2) of P. multiforum extract of crude plant**

**Table 2. XRD parameters of Phyto-synthesis (CdO NPs) Nano-particles using procedure (1 and 2) of "P. multiforum "extract of crude plant**

| Sample | Planes (hkℓ) | 2 theta (DEG) | FWHM (DEG) | D (nm) | STRAIN XE-4 | DIS X1014 |
|---|---|---|---|---|---|---|
| **Procedure 1** | **200** | **38.250** | **0.700** | **11.95** | **116** | **70** |
| **Procedure 2** | **200** | **38.28** | **0.600** | **13.95** | **99.38** | **51.42** |

FWHM is the Full width at half maximum; D: dimension of Crystal in nm; η $x10^{-4}$: strain value; δ x $10^{14}$: dislocation density; NPs: Nano-particles; BPs: particles.

## CONCLUSIONS

"P. multiforum "extract of crude plant contain five flavonoids in its leaf (Emodin, followed by Aloe -emodin, Chrysophanol, Rhein and Physcion). CdO Nano-particles were produced using two different techniques. Every technique used to produce CdO at the nano--scale was successful. Examining the role that specific active "P. multiforum" compounds play in the phyto-synthesis of CdO Nano-particles will be helpful.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## DECLARATION OF FUND

The authors declare that they have not received a fund.

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