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Green Synthesis and Characterization of ZnO Nanoparticles using *Calabash extract*

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ABSTRACT

This is the most preferred method of preparation as it makes use of pollution free chemicals and encourages the use of non-toxic solvents such as water and plants extracts. The present study is proposed with an objective to synthesize ZnO nanoparticle by the eco-friendly green synthesis using environmentally benign extract of *calabash* (*Crescentia cujete*) where zinc nitrate acts as the precursor. The synthesized ZnO nanoparticles were characterized by X-ray diffraction (XRD), Transmission electron microscopy (TEM), Field emission scanning electron microscopy (FE-SEM) and Energy dispersive spectroscopy (EDS).

Key words: *Crescentia cujete*, ZnO nanoparticle, X-ray diffraction, Eco-friendly green synthesis

Virtually, all parts of the calabash (*Crescentia cujete*) tree have been found to be useful; the wood for tool handles, ribs in boat building, cattle yokes, and the gourd is used for cups, containers and musical instruments. The calabash (*C. cujete*) fruit was studied for its chemical constituents-proximate and mineral composition as well as phytochemical properties. The sugar content, energy content, electrical conductivity and pH of the fruit were also determined. The value of the fat, protein, nitrogen, crude fibre, moisture content, sucrose, fructose, galactose and energy content are quite high viz. 1.13, 8.35, 1.34, 4.28, 84.92, 59.86, 25.09, 18.24 and 88.69%, respectively. The pH of the fruit falls within the acidic range (4.80) and the mean value recorded for the electrical conductivity was 163.24 µS/cm. The results obtained for the mineral elements show that sodium and phosphorus have high mean concentrations, while low mean concentrations were recorded for others. Also, the results show relatively low mean concentrations for the heavy metals; but high mean concentrations for manganese, iron, zinc and copper. The presence of phytochemicals like saponins, flavonoid, cardenolides, tannins and phenol as well as the presence of hydrogen cyanide were observed in the fruit sample. The findings on the phytochemical constituents, mineral composition and proximate composition of the *C. cujete* suggest that the fruit can make useful contribution to both human and animal nutrition and possesses medicinal values.

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The use of plants for remedies has long been in existence and is among the most attractive sources for developing drugs [1]. Any part of plant can be considered as herbs including leaves roots, flower, seeds, resins, leaf sheath, bark, inner bark (cambium), berries and sometimes the pericarp or other portion [2]. Most primates depend heavily on the leaves, fruits, and flowers of tropical plants to meet their nutritional demands [3-5]. As a result, the chemical composition of these plant parts is critical to understanding primate ecology and evolution. These ancient indigenous practices were discovered by series of 'trial and error' which then could not be substantiated by proven scientific theories. Fruits are rich with antioxidants that can prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species [6]. The most abundant antioxidants in fruits are polyphenols and vitamins C, A, B and E; while carotenoids are present to a lesser extent in some fruits. These polyphenols with antioxidant activities are mostly belong to flavonoids [7].

The Calabash tree, scientifically known as *Crescentia cujete*, has been used by indigenous system of medicine to treat several illnesses. The pulp of fruit has medicinal properties and acts as remedy for respiratory problems such as asthma and cough. The leaves are used to reduce blood pressure. The decoction of tree bark is used to clean wounds and also to treat hematomas and tumors. Because of this, Calabash tree is considered to be a miracle fruit [8]. On the other hand, according to Thewlis and Meyer [9] rats are becoming increasingly popular as small laboratory for hematologic studies, since blood is easily obtained in adequate quantities for routine counts. There is considerable fluctuation in the leukocytes particularly, but the instability seems to be no greater than that of guinea pigs or rabbits. Rats are less expensive than either, and blood is more easily obtainable from them than from guinea pigs. Very few literatures have been explored as to the potential of its fruit pulp ethanolic extract for organogenesis in female rat vertebrate model and its overall hematological effects in gestating rats, as blood parameters are sensitive indicators to an organism's physiological state [10].

The Mayan civilization became vastly prominent in southern Mexico and other South American counties around 2000 BC. The tropical regions inhabited were an ideal environment for parasites and other infectious bacteria [11]. When diseases occurred, local Mayan healers often took advantage of the variety of medicinal plant resources provided in the local rain forest [12]. Mayan healers used herbal remedies to cure many different diseases, often relating the disease with the plant to be used. For example, red plants were used for rashes, blood disorders, and burns; blue plants were used for neural disorders; and yellow plants were associated with the liver and spleen. Often white plants were avoided because white was associated with death [13]. Calabash Crescentia cujete L. (family Bignoniceae) is a tree found in the West Indies, tropical America, and tropical areas of the Old World [14]. Calabash blooms during the month of June and its fruits grow and ripen slowly as they remain on the tree for six to seven months. During this time, the fruit changes from green to yellow and is harvested during the dry season from December to May [15]. The hard outer shell of the calabash fruit has been used for food containers, bowls, tobacco pipes, and as musical tools [17]. The white spongy pulp inside the shell contains numerous flat seeds. The fruit itself can be mixed with milk, heated, and consumed for treating colds and asthma [17]. Ripe fruit has also been regarded as a laxative [18]. Traditional Mayan healers have recommended that the fruit may be ingested to force menses, birth, after birth [19-20] or trigger abortions [21]. The latter has been observed in cattle [22-23].

Disease has been an integral part of man from the beginning of his existence. The subject of drugs is also as old as disease and the search for remedies to combat it is perhaps equally old and for more than a millennium, herbal medicine has been extensively used, apparently safely and effectively to alleviate various symptoms of disease [24]. The most important bioactive compounds of plants are alkaloids, flavonoids, tannins, glycosides and phenolic compounds [25-26]. These compounds possess numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic, antithrombotic and vasodilatory activities [27]. Inflammatory diseases including different types of rheumatic diseases are very common throughout the World [28]. Although, the rheumatism is one of the oldest known diseases of mankind and affects a large population of the world and no substantial progress has been made in achieving a permanent cure. Non-steroidal anti-inflammatory drugs (NSAIDs) are used throughout the world for the treatment and management of inflammation, pain and fever. The use of NSAIDs, however, has not been therapeutically successful in all conditions of inflammation [29]. Moreover, adverse effects associated with NSAIDs can lead to ulcers and hemorrhage. The expanding bacterial resistance to antibiotics has become a growing concern worldwide [30]. Intensive care physicians consider antibiotic-resistant bacteria a significant or major problem in the treatment of patients [31]. Increasing bacterial resistance is a prompting resurgence in research of the antimicrobial role of herbs against resistant strains [32]. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds [33]. As an alternative, plant-based medicines are getting an increased therapeutics market share due to their mild action and fewer adverse effects. According to the World Health Organization nearly 80 % of the world population prefers plant-based drugs [34]. The research on screening and development of drugs for their activity is therefore, an unending process and there is hope of finding out anti-inflammatory drugs from indigenous plants. Various plant extracts and their isolated compounds have been proved as good as synthetic anti-inflammatory agents [35]. Latest and previous studies have concluded the beneficial aspects of plant derived drugs as good source of antibiotics, antioxidants and anti-inflammatory agents [36-37]. The present study was designed to investigate the TPC and TFC and to evaluate the anti-inflammatory and antibacterial activities of ethanol extract and fractions of leaves and bark of *C. cujete*. *C. cujete* tree belongs to the family of Binoniacea. It is also known as the gourd tree or calabash tree. The tree is about 6–10 m tall with a wide crown and long branches covered with clusters of tripinnate leaves and gourd-like fruit. The branches have simple elliptical leaves clustered at the anode. According to folk medicine, the fruit pulp is used for respiratory problems such as asthma and also used as laxative. The bark is used for mucoid diarrhea. Bark decoction is used to clean wounds and pounded leaves are used as poultice for headaches. Internally, the leaves are used as diuretic and in the treatment of hematomas and tumors. Fruit decoction is used to treat diarrhea, stomachaches, cold, bronchitis, cough, asthma, and urethritis. The leaves on the other hand are used for the treatment of hypertension [38]. The juice from fruits mixed with sugar and bee's honey are eaten for the purpose of solving the problems of the respiratory system (asthma, catarrh), the digestive system (stomach pains, intestinal parasites) and female reproductive apparatus (infertility) [39]. DPPH radical scavenging, antioxidant activity by β-carotene bleaching test and cytotoxic activity of the methanol extract of aerial parts of this plant were evaluated by Juceni *et al*. [40]. It has been reported that ethanol extract of *C. cujete* leaves showed significant antibacterial activity against *Shigella dysenteriae*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium* and *Staphylococcus aureus* [41].

Green synthesis of zinc oxide nanoparticles using extract of calabash (*Crescentia cujete*) and (Fig 1). Show the photograph of the calabash (*Crescentia cujete*).

Fig 1 The photograph shows the *calabash* (*Crescentia cujete*)

MATERIALS AND METHODS

Zinc nitrate hexahydrate ($Zn(NO_3)_2$. $6H_2O$), distilled water and extract of *calabash* (*Crescentia cujete*) is used for the preparation ZnO nanoparticle.

Preparations of aqueous extract from moringa oleifera leaf

Calabash (*Crescentia cujete*) was collected from the campus of Thanthai Roever Institute of Agriculture and Rural Development, Perambalur, Tamil Nadu, India. The calabash (*Crescentia cujete*) were identified and authenticated by the Department of Botany, Annamalai University, Chidambaram, Tamil Nadu, India. The collected calabash (*Crescentia cujete*) samples were washed with double distilled water to remove impurities.

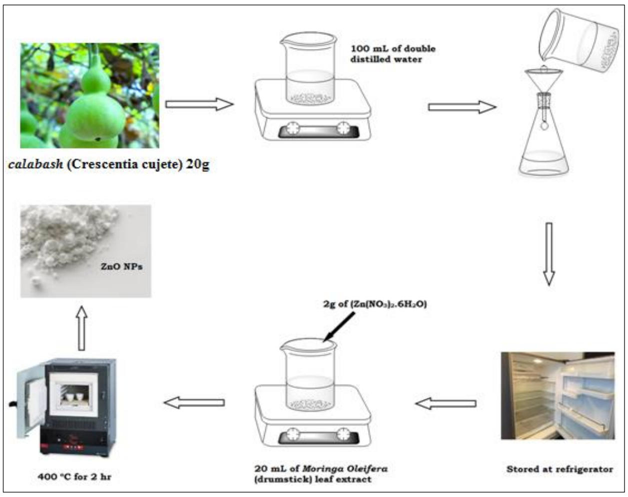

Fig 2 Flow chart shows the synthetic route of ZnO nanoparticles

Green synthesis of ZnO nanoparticles using calabash (Crescentia cujete) extract

The collected calabash (*Crescentia cujete*) were washed thoroughly 2–3 times with running tap water and sterilized with double distilled water. The leaves sample was allowed to dry in room temperature (32ºC) and 20g was taken for synthetic purpose. 20g weighed calabash (*Crescentia cujete*) were boiled with 100 mL of double distilled water for 20 min at 80ºC. During the procedure of boiling, a light-yellow colored solution was formed and it was cool at room temperature. After that, the yellow-colored extract was filtered with filter paper (Whatman No.1) and stored in refrigerator until further use. Further, 20 mL of calabash (*Crescentia cujete*) extract was taken from the stock solution (stored at refrigerator) and boiled at 60–80ºC. When the temperature of the solution reached at 60ºC, 2g of zinc nitrate hexahydrate crystals ($Zn(NO_3)_2.6H_2O$) was added. The mixture was boiled until the formation of yellowish-brown colored paste. The paste was transferred to a ceramic crucible cup and heated in muffle furnace which was maintained at 400 ºC for 2 hrs. The obtained white colored powder was used for structural and optical studies. The flow chart used for the preparation of ZnO nanoparticle is shown in (Fig 2).

Characterization techniques

The X-ray diffraction (XRD) patterns were obtained for the centrifuged and dried samples using X–ray Rigaku diffractometer with Cu K_α source (30 kV, 100 mA), at a scan speed of 3.0000 deg/min, a step width of 0.1000 deg, in a 2θ range of 20-80̊. Transmission electron microscope (TEM) was recorded using a JEOL JEM 2100 high resolution transmission electron microscope (HR-TEM) at an accelerating voltage of 200 kV. The samples were dispersed in acetone and spread on the grid for imaging. Formavar coated copper grids were employed to load the sample. The energy dispersive X-ray (EDS) spectra of the nano semiconductors were recorded with a JEOL JSM-5610 scanning electron microscope (SEM) equipped with back electron (BE) detector and EDX. The sample was placed on an adhesive carbon slice supported on copper stubs and coated with 10 nm thick gold using JEOL JFC-1600 auto fine coater prior to measurement. The presence and interaction of chemical functional groups was analyzed using FT-IR spectrophotometer (Perkin Elmer) at the range of 4000-400 cm^{-1}.

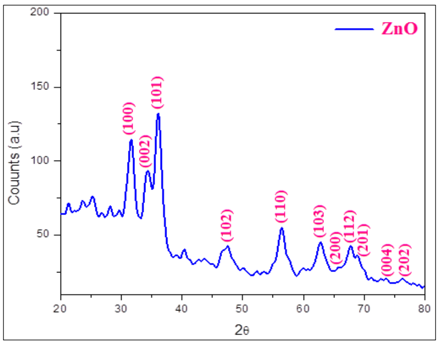

Fig 3 XRD spectrum of ZnO nanoparticles

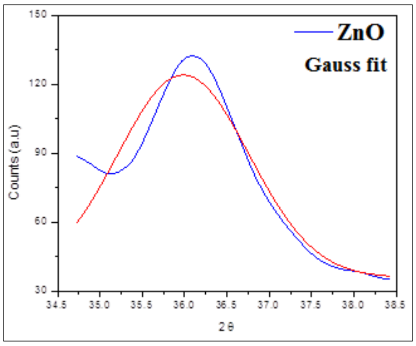

Fig 4a XRD – Gauss fit parameter of ZnO nanoparticles

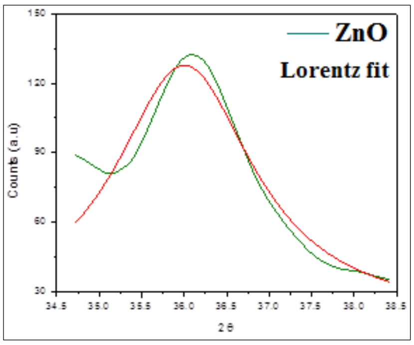

Fig 4b XRD - Lorentz fit parameter of ZnO nanoparticles

RESULTS AND DISCUSSION

XRD analysis of ZnO nanoparticles

The XRD technique uses the scattered intensity of an X-ray beam on the sample, revealing information about the crystallographic structure, chemical composition and physical properties of the material studied. The XRD pattern of ZnO nanoparticles (Figure 3) recorded in the range of 20° to 80°, with a scanning step size 0.02°. All diffraction peaks attributed to crystalline of ZnO with the hexagonal primitive structure (space group: P63mc (186); a=3.249 nm, c=5.206 nm). The data obtained are in good agreement with the joint committee on powder diffraction standards card for ZnO (JCPDS 89-7102). The peak at 2θ values 31.72, 34.32, 36.12, 47.37, 56.47, 62.62, 65.73, 67.67, 68.87, 73.57 and 76.32 is corresponding to the plane of (100), (002), (101), (102), (110), (103), (112), (201) and (202) reflections respectively. The average crystallite sizes (L) of the sol–gel synthesized ZnO have been deduced as 20 nm. They have been obtained from the full width at half maximum (FWHM) (Figure 4a&b) of the most intense peaks of the respective crystals using the Scherrer equation, L = 0.9 $\lambda/\beta\cos\theta$, where λ is the wavelength of the X-rays used, θ is the diffraction angle and β is the full width at half maximum of the peak, the calculated surface area for ZnO 28.73 m^2/g.

Transmission electron microscopy (TEM) analysis

(Fig 5) show TEM image of biosynthesized ZnO nanoparticles. The image reveals spherical shape of ZnO nanoparticles which are in accord with XRD. It is observed that the ZnO nanoparticles tended to agglomeration due to high surface energy that usually occurs when synthesis is carried out in aqueous medium and also possibly due to densification resulting in narrow space between particles [42]. The average particle size measured by TEM image is observed to be 20 nm (Fig 6) which is in good agreement with the crystallite size calculated from XRD analysis with some deviation due to measurement base on difference between visible grain boundaries (TEM) and diffracts X-ray coherently in extended crystalline region (XRD). The image clearly shows the presence of secondary material capping which may be assigned to bio-organic compounds present in the leaf extract that confirm by the observing sharp Bragg's reflection in the XRD spectrum [43].

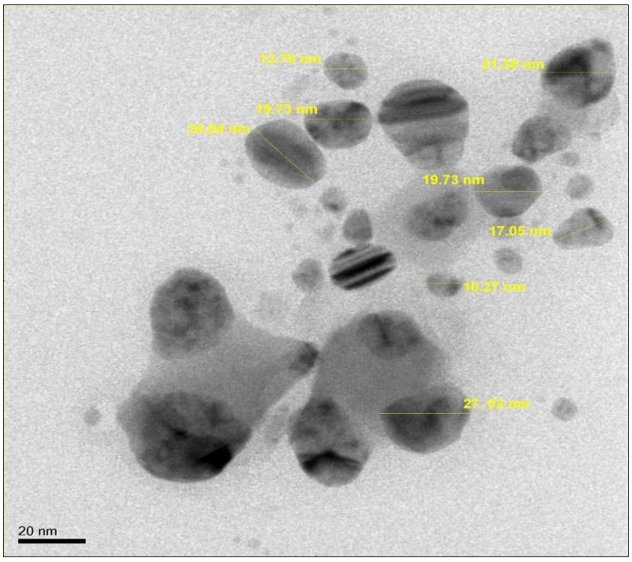

Fig 5 TEM image of ZnO nanoparticles

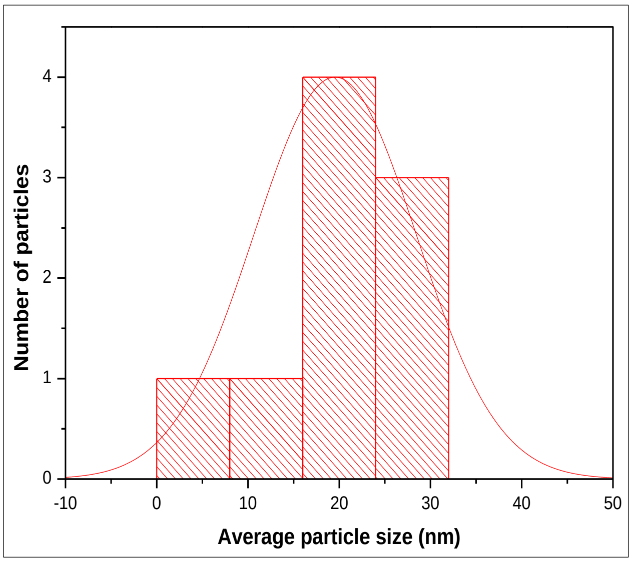

Fig 6 TEM image of corresponding particle size distribution histograms

FE-SEM with EDX analysis

FE-SEM image provides the information on morphology, topography and size of the particle. The synthesized ZnO nanoparticle seen to be homogenous, agglomerated and without the presence of the other dominating phases [44]. This is due to the high surface energy of the individual ZnO nanoparticles.

The FE-SEM images (Fig 7) shows crystal like morphology with a self-aligned nanoparticle. The size and structure of the ZnO nanoparticle are in good agreement with XRD result with some deviation. EDX spectrum (Fig 8) shows purity and composition of the green synthesized ZnO nanoparticle. From the EDX spectrum strong signal is observed from Zn element and light signals from O, Mo, Cl, K and Ca elements. These weak signals are due to the X-ray emission from the macromolecules like protein, enzymes, carbohydrate present in the cell wall of the plant leaf extract.

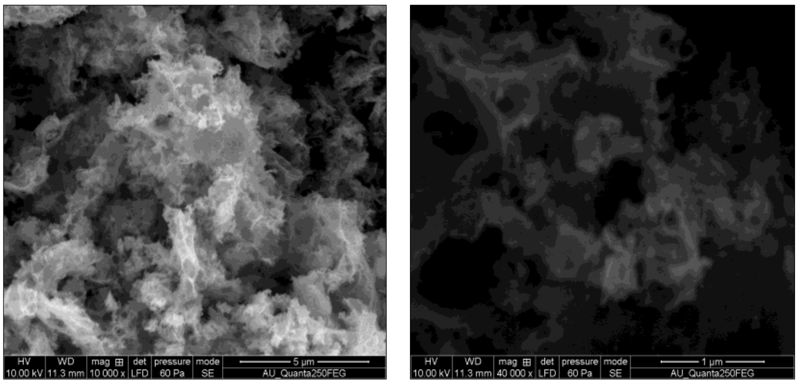

Fig 7 FE-SEM images of green synthesized ZnO nanoparticles at different magnifications

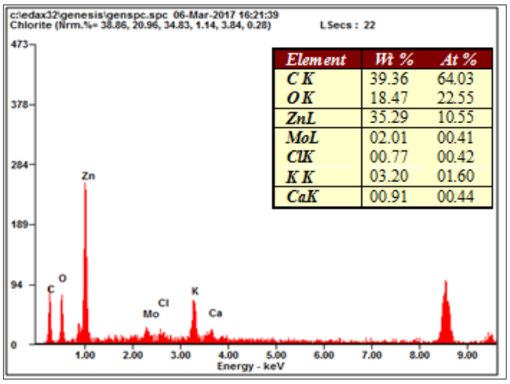

Fig 8 EDX spectrum of green synthesized ZnO nanoparticles

CONCLUSION

Zinc oxide nanoparticles is synthesized by green synthetic method by using calabash (*Crescentia cujete*) extract. Diffraction peaks observed in the XRD spectrum reveals the hexagonal primitive structure and confirmed with the values from JCPDS data. Size of the nanoparticles is dependent on the annealing temperature and the value of the size of the nanoparticle is in the range of 20 nm. The FE-SEM showed distinguished spherical morphology with a self-aligned ZnO nanoparticles. EDX spectrum confirmed purity and chemical composition of the green synthesized ZnO nanoparticles. Moreover, present work on synthesized ZnO nanoparticles using the green method synthesis important advantages such as lesser or almost zero contamination in the environment because they are derived from the natural plant leaf extract.

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