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PIPER BETEL L. ASSISTED CeO_2 NANOPARTICLES: PHYTOCHEMICAL SCREENING AND SPECTRAL INVESTIGATIONS

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ABSTRACT

Biosynthesized CeO_2 NPs, using aqueous leaf extract of *Piper betel L.* have been used as the reducing agent. Synthesised CeO_2 nanopartclies were subjected to Thermal (TG - DTA), Structural (XRD) and Morphological (SEM) analyses. The calcination temperature was fixed at 850 ºC. The obtained nanocrystals were found to be cubic with crystallite size being 49 nm. The aqueous *Piper betel L.* leaf extract, as - prepared Cerium oxide nanopowder and biosynthesized CeO_2 nanoparticles were subjected to qualitative phytochemical analysis as well as the spectral analyses like FTIR, GC - MS and ^{1}HNMR. In all three cases, the phytochemical examination revealed the existence of phenols, coumarins, glycosides, carbohydrates and terpenoids. The existence of phenols and anthraquinones was verified by FTIR spectral investigations. In the current case of GC - MS, flavonoids, phenols, and hydrocavicol were found to be present. In the case of ^{1}HNMR, the availability of phenols, in all the three cases, has been detected. Therefore, the results of the present study confirmed the pivotal role played by phenols in the formation of *Piper betel L.* induced CeO_2 nanoparticles.

Keywords: *Piper betel L.,* CeO_2 NPs, phytochemicals, FTIR, GC - MS and ^{1}HNMR

INTRODUCTION

Piperaceae represents the family of *Piper betel L.,* and it is typically found in hot, humid climates. It is regarded as one of the most beneficial herbal remedies and its leaves were used for various therapeutic applications. Many tribes in India still use it to treat and guard against various illnesses. According to a preliminary investigation, *Piper betel L.* leaf extract contains a significant amount of bioactive compounds [1, 2]. Aqueous crude extract of *Piper betel L.* has been shown to have efficacy against the bacteriae [3]. A wide range of medicinal properties has been reported to be exhibited by *Piper betel L.* [4]. In addition to immunological modulatory, gastroprotective and anti-diabetic properties, the pharmacological profile has been found to have antiplatelet and anti-inflammatory actions [5]. Use of *Piper betel L.* in wound dressing and wound healing was known to Indian communities [6].

Photochemicals are, Plants that contains naturally occurring chemicals that are physiologically active as well as offer nutrition and medical advantages to people [7].These phytochemicals don't have any negative effects, in contrast to pharmaceutical compounds. More than 4,500 phytochemicals have been identified to date and they are categorised according to their protective properties and physical and chemical makeup. About 350 of these photochemicals have undergone in-depth research [8].

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With the chemical formula CeO_2, cerium oxide is a light yellow-white powder with a number of unique characteristics, including a high refractive index, a high dielectric constant, and a lattice constant that is almost identical to silicon. Numerous uses for cerium oxide may be found in microelectronics and optics. Cellular ageing is prevented by cerium nanoparticles [9]. Researchers have discovered that nanoceria particles have anti-inflammatory and antioxidant effects. Neurodegenerative illnesses including Parkinson's and Alzheimer's disease may be treated using cerium oxide nanoparticles [10, 11].

This paper describes the environmentally friendly manufacturing of CeO_2 nanoparticles utilising *Piper betel L.* leaf extract in this context. Various chemical constituents available were identified using qualitative phytochemical analysis and spectral techniques.

MATERIALS AND METHODS

Piper betel L. leaves were collected and used as a bioagent. These leaves were collected in and around Chidambaram Town, Cuddalore district, January 2022 in the Indian state of Tamil Nadu. Cerium (III) sulfate [$Ce_2 (SO_4)_3$] was procured from M/s Sigma Aldrich (Mumbai, India), as well as used as a precursor in the present work. Dry spectral grade KBr (98.7% purity) was used to prepare the pellet to carry out the FTIR analysis. AR grade chemicals like Sodium nitrite, Hydrochloric acid, Sodium hydroxide, Sulphuric acid, Glacial acetic acid, Ferric chloride, Ninhydrin, Iodine solution and Chloroform were acquired from M/s Merck (Mumbai, India). DCM (Dichloromethane) solvent was procured from M/s Sigma Aldrich (Mumbai, India) and used for the sample preparation for GC - MS analysis. DMSO (Dimethyl sulfoxide) solvent was procured from M/s Sigma Aldrich (Mumbai, India) and used in the case of NMR analysis.

PREPARATION OF PLANT EXTRACT

After being properly cleansed with deionized water to remove any dust, the collected *Piper betel L.* leaves were shade dried. Afterwards, they were ground well into a fine powder. 100 ml of distilled water was added to 10 g of leaf *(Piper betel L.)* powder. The suspension was heated for four hours at 60 °C before being cooled at room temperature. The extract of the leaf (*Piper betel L.*) was obtained by filtering the solution using Whatman No. 1 filter paper [12].

PREPARATION OF CEO_2 NANOPARTICLES

2.5 g of precursor [$Ce_2 (SO_4)_3$] was dissolved in 40 ml of deionized water to prepare a 0.1M solution. 20 ml of the prepared leaf extract was added to the precursor solution, which was then heated at 60 ^oC for 4 hours. The combination was then heated until it reached a jelly-like state, and the jelly-like state was then subjected to a four hour calcination process at 850 ^oC in a muffle furnace to create white CeO_2 nanoparticles [12]. The obtained nanosample was then preserved in vials for further analysis.

Fig. 1 represents the procedure involved in the preparation of biogenic CeO_2 nanosample.

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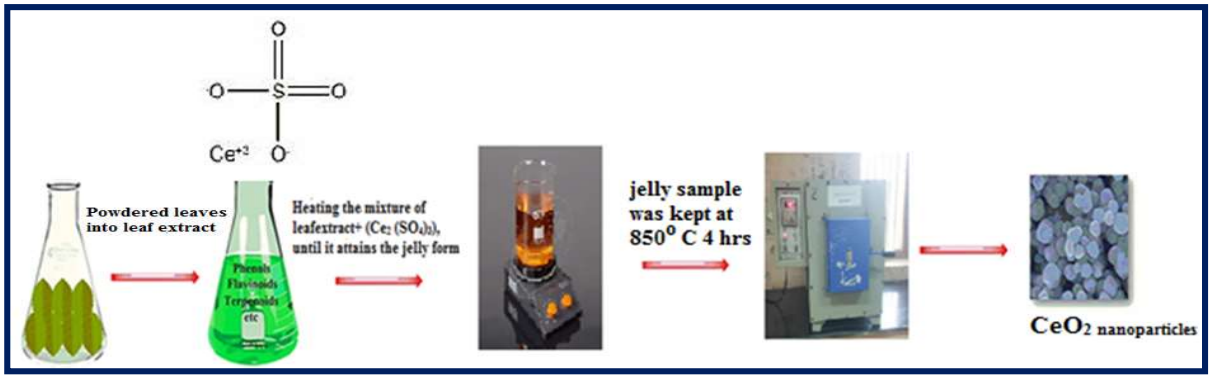

Fig. 1 Procedure for preparation of *Piper betel L.* leaf mediated CeO_2 nanoparticles

INSTRUMENTS

The NETZSCH-STA 449F3-JUPITER analyzer was used to conduct the TG-DTA analysis, with temperature range from 25 °C to 1000 °C. The XRD spectrum was recorded using a FEI-TECHNAI G2-20S twin X-ray spectrometer with 2θ in the range of 20° to 80°. The morphology of the biosynthesized CeO_2 nanoparticles was investigated by employing ZEISS EVO 18 Scanning electron microscope. The elemental composition of the bioinspired CeO_2 nanosample was examined using an Oxford Energy Dispersive X-ray system (INCA 250 EDS with X-MAX 20 mm Detector). The Perkin Elmer L1600300 Spectrum Two LiTa system was employed in the present work for recording the FTIR spectrum. The qualitative phytochemical analysis was done by adopting appropriate test procedures [13 - 15]. GC - MS investigations were executed by utilizing Perkin Elmer GC model Clarus 680 with Clarus 600 mass spectrometer. A 400 MHZ, BRUKER AVANCE I, NMR spectrometer was used to investigate the samples for NMR studies.

RESULTS AND DISCUSSION

A. THERMO GRAVIMETRIC ANALYSIS / DIFFERENTIAL THERMAL ANALYSIS

The thermal characteristics of the as - prepared sample of CeO_2 nanoparticles were studied by using TG - DTA technique, as described earlier and represented as Fig. 2.

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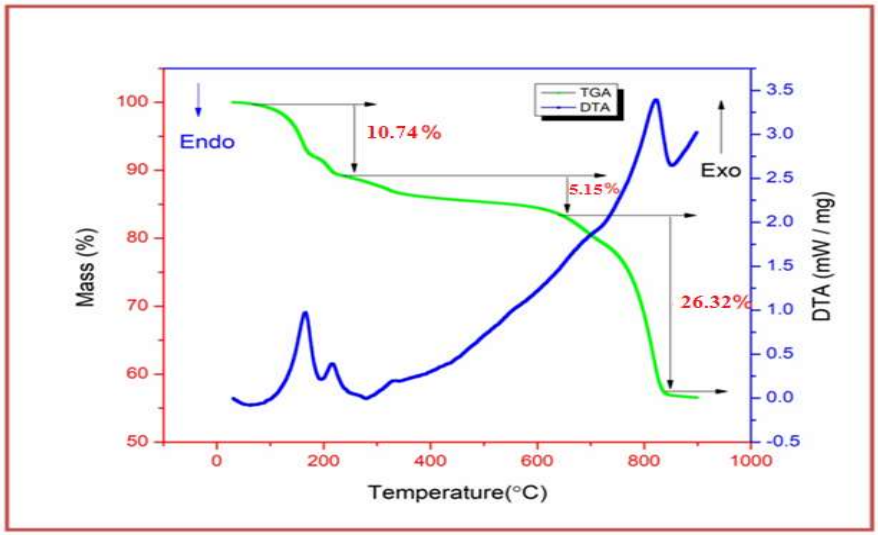

Fig. 2 TG - DTA curve for as-prepared CeO_2 nanopowder using *Piper betel L.* leaf extract

From Fig.2 it is obvious that the first stage of weight loss (≈ 10.74 %) occurred, with an endothermic peak, at 225.94 °C which might be because of the removal of locally bound water molecule. The second weight loss of approximately 5.15 %, induced by decomposition of $Ce_2(SO_4)_3$, occurred around 635.27 °C. The third and final stage of weight loss of about 26.32 % was recorded between 826 °C and 900 °C. This weight loss may be responsible for the decomposition of chemically bound chromophoric groups and the residual organic constituents of the precursor. These thermal variations may also be due to the presence of organic phase phytochemicals adsorbed on the surface of the synthesized CeO_2 nanopowder. Thirunavukkarasu et al. (2020) and Gopinath et al. (2015) have detected and reported similar weight losses for the synthesized CeO_2 nanosamples [16, 17]. The thermal stabilization has been obtained at 826 °C and hence, the as - synthesized sample was calcined at 850 °C. After calcination, the sample was ground well to get fine powdered *Piper betel L.* induced CeO_2 nanopowder and stored for further analysis.

B. STRUCTURAL ANALYSIS

Fig. 3 represents the XRD spectrum recorded for the *Piper betel L.* induced Cerium oxide nanoparticles.

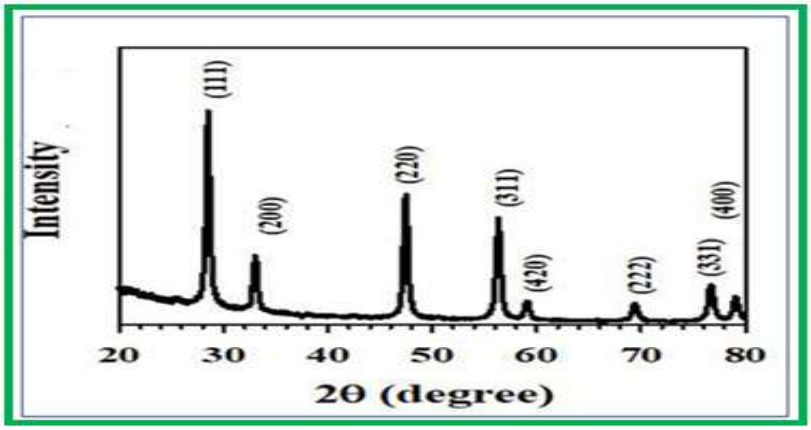

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Fig. 3 XRD pattern of bio-Prepared Cerium oxide using *Piper betel L.* leaf extract

Prominent peaks were observed at 27.93 º, 32.47 º, 46.89 º, 55.72 º, 58.47 º, 68.78 º, 76.07 º and 78.46 º representing the crystal planar orientations (1,1,1), (2,0,0), (2,2,0), (3,1,1), (4,2,0), (2,2,2), (3,3,1) and (4,0,0) correspondingly. On comparison with JCPDS data [card number (34-0394)], it has been noticed that the CeO_2 nanoparticles, as synthesized in the present study, exhibited cubic crystal structure. Using Debye-Scherrer's formula, various crystallographic data have been calculated and tabulated (Table - 1)

TABLE - 1

XRD data for the biosynthesized CeO_2 NPs

Position (2θ)	**(h k l)**	**FWHM**	**Integrated Int. (Counts)**	**d spacing (Å)**	**Lattice constant (a)**	**Crystallite size (D) [nm]**	**Micro strain (Є) [x 10^{-3}] (Ɛ)**	**Dislocation Density (δ) [x 10^{15} m^{-2}]**
27.9256	(1 1 1)	0.1968	100	3.195	1.263	42.290	0.856	5.591
32.4704	(2 0 0)	0.1476	27	2.757	2.255	56.388	0.642	3.145
46.8883	(2 2 0)	0.1968	61	1.937	3.433	42.916	0.856	5.429
55.7213	(3 1 1)	0.2952	46	1.649	3.101	28.194	1.284	1.257
58.4705	(4 2 0)	0.1968	8.52	1.578	8.458	42.292	0.856	5.590
68.7822	(2 2 2)	0.1476	9.00	1.364	6.766	56.390	0.642	3.144
76.0761	(33 1)	0.1200	19. 05	1.250	13.178	69.361	0.521	2.078
78.4561	(4 0 0)	0.1476	10. 41	1.219	9.022	56.391	0.642	3.144
					Mean	49.454 ± 1.172	0.749 ± 0.144	

From Table - 1, the average 'D' and 'Є' values were found to be 49 nm and 0.75, respectively. Using W - H plot method (Fig . 4), the 'D' and 'Є' values were found out and

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compared with those obtained using Debye - Scherrer's formula (Table - 2). Table - 3 portrays the outcome of the present XRD investigation with those reported by the earlier workers [18 - 21].

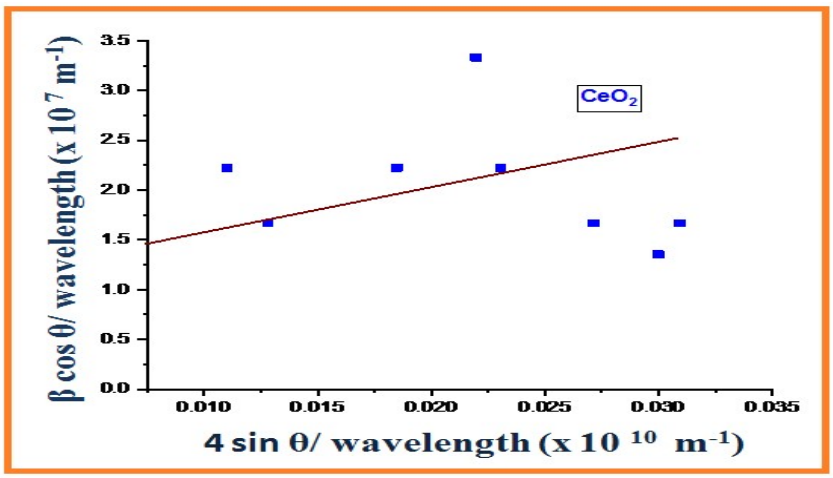

Fig. 4 Williamson - Hall plot of biosynthesized CeO_2 NPs using leaf extract

TABLE - 2

Comparison of Debye - Scherrer's and W - H methods

Method	Crystallite size (D) [nm]	Micro strain (Є) [x 10^{-3}]
a) Scherrer's formula	49 ± 1.17	0.75 ± 0.14
b) W - H Plot	30	0.017 ± 0.002

TABLE - 3

Comparison of the results of XRD analysis

S. No.	Metal oxide synthesized	Precursor used	Method	Crystallite size (D) [nm]	Reference
1	Cerium oxide	Ammonium cerium (IV) nitrate	Green synthesis	27	Das et al., 2022 [18]
2	Cerium oxide	Cerium nitrate hexahydrate	Hydrothermal	14	Palard et al., 2021 [19]
3	Cerium oxide	Cerium nitrate hexahydrate	Green synthesis	30	Ahmed et al., 2021 [20]
4	Cerium oxide	Cerium nitrate hexahydrate	Sol - gel	8	Choudhury et al., 2012 [21]
5	Cerium oxide	Cerium (III) sulfate	Green synthesis	49	**Present work**

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C. MORPHOLOGICAL ANALYSIS

Using the SEM technique, the surface morphology of the synthesised nanoparticles was examined. The outcomes have been displayed in Fig. 5.

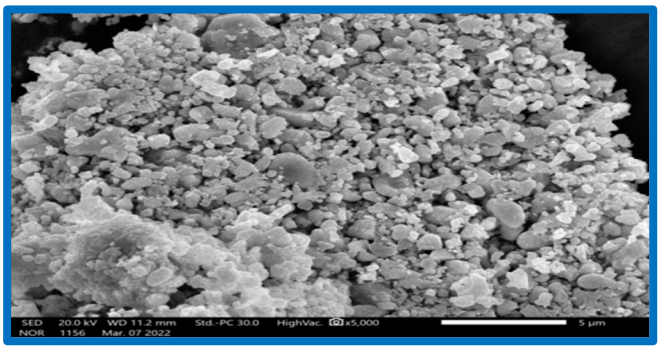

Fig. 5 SEM image of *Piper betel L.* mediated CeO_2 nanoparticles

A close scrutiny of the Fig. 5 reveals the fact that the biosynthesized CeO_2 nanosample exhibited well - defined cubic shape. The results of observation are in good accord with XRD analysis. Similar observations, pointing out the cubic shape for the CeO_2 nanoparticles, were noted by Lulu He et al., 2019 [22] and Liu et al., 2015 [23].

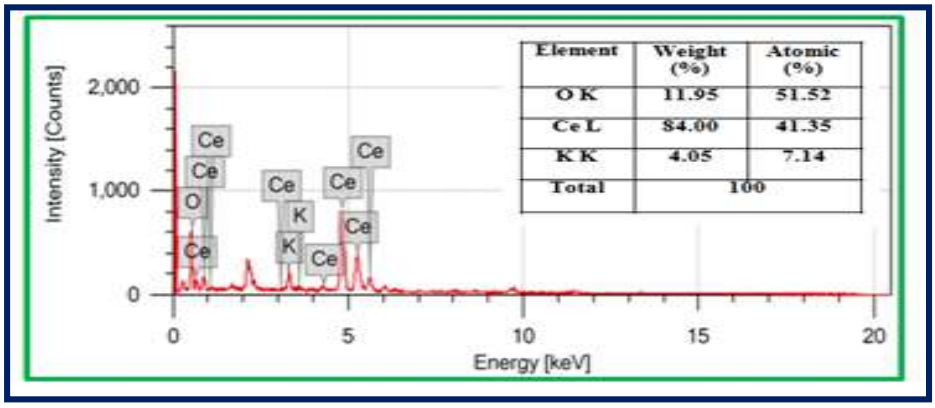

Fig. 6 EDAX spectrum of the biosynthesized CeO_2 nanoparticles

Fig. 6 represents the EDAX spectrum recorded for the *Piper betel L.* assisted cerium oxide nanoparticles. From Fig. 6, it is quite obvious that the prepared nanosamples consisted of Ce, O and K. The presence of K in the prepared biomaterial may be of plant origin. Considering the percentage composition of Ce (41.35 %) and O (51.52 %), the percentage composition of K (7.14%) may be neglected and hence, the results of EDAX analysis indicated the high amount of purity associated with the *Piper betel L.* induced CeO_2 nanosample. Earlier workers [Kashyap et al., 2022 and 2023] have also reported similar results indicating the presence of Ce and O [24, 25].

D. FTIR SPECTRAL ANALYSIS

In order to investigate for the available bioactive compounds, the aqueous extract of *Piper betel L.* leaves, as - synthesized (before calcination) and biosynthesized (after calcination) CeO_2 nanosamples were subjected to FTIR analysis and the spectra were taken in the region of 4000 -

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400 cm^{-1}. Fig. 7 (a) represents the FTIR spectrum recorded for *Piper betel L*. leaf extract. Table - 4, shows that the various functional groups that were present in the sample have been identified.

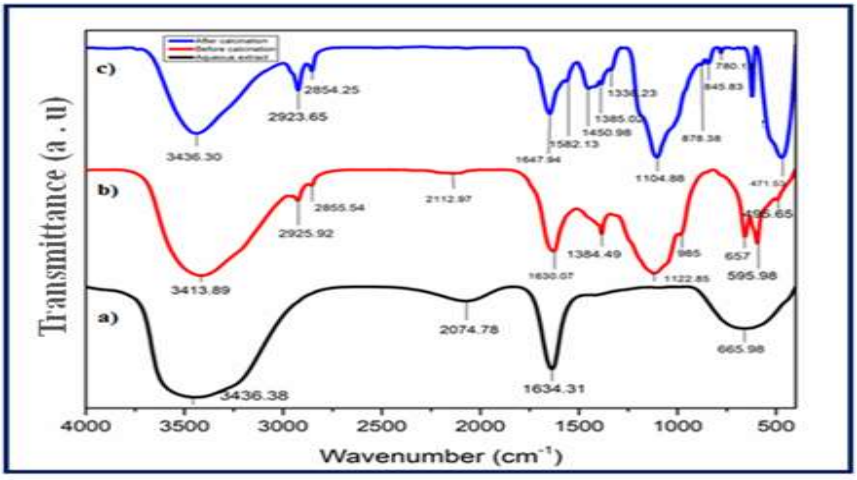

Fig. 7 FTIR Spectrum for (a) crude extract of *Piper betel L.* (b) as - synthesized nanoparticles (before calcination) and (c) bioprepared CeO_2 nanoparticles (after calcination)

Table - 4

Tentative assignments for the FTIR peaks of crude *Piper betel L.* leaf extract

S. No.	PEAK VALUE (cm $^{-1}$)	Assignment
1	3436	O - H Stretching (Phenol)
2	2074	C - H bond (Aromatic compound)
3	1634	C = O (Anthraquinones)
4	665	M - O Stretching

A close examination of Table - 4 indicates the presence of functional groups associated with various phytochemicals, such as phenols and aromatic compounds, as well as anthraquinones and metal oxide. Fig. 7 (b) represents the FTIR spectrum recorded for the as - synthesized (before calcination) CeO_2 nanosample synthesised via *Piper betel L*. leaf extract. Table - 5 represents the tentative assignments made. From Table - 5 it is clear that, in addition to the functional groups pertaining to phytochemicals like phenol, alkaloids, anthraquinones, saponins and vitamins, the Ce - O - Ce band has also been detected.

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TABLE - 5

Tentative assignments for the FTIR peaks of as - synthesized nanoparticles (Before Calcination)

S. No.	PEAK VALUE (cm^{-1})	Assignment
1	3413	O - H Stretching (phenol)
2	2925	Alkyl chain (alkaloids)
3	2855	Alkyl chain (alkaloids)
4	1630	C = O (Anthraquinones)
5	1122	C - F alkyl halide (vitamins)
6	985	C - O Stretching
7	657	C = Cl alkyl halide (acids/ Saponins/ steroids)
8	595	Phonon mode of Ce - O – Ce
9	495	Formation of Ce - O - Ce bond

FTIR spectrum was also recorded for the *Piper betel L*. mediated biosynthesized CeO_2 nanoparticles (after calcination) and the existence of several functional groups was identified and reported, here [Fig. 7 (c) and Table - 6].

TABLE - 6

Tentative assignments for the FTIR peaks of bioprepared CeO_2 nanopowder (After Calcination)

S. No.	PEAK VALUE (cm^{-1})	Assignment
1	3436	O - H Stretching vibration (Phenol)
2	2923	Alkyl chain (alkaloids)
3	2854	Alkyl chain (alkaloids)
4	1647	C = O (Anthraquinones)
5	1104	C - O Stretching
6	845	= C = H alkene (lignins)
7	780	Ce - O - Ce bond
8	471	Formation of O - Ce - O bond

The FTIR spectrum for biosynthesized CeO_2 NPs depicted the strongest peak at 3436 cm^{-1}, likely due to the stretching vibrations of OH (phenols). The absorption peak at 2923 and 2854 cm^{-1} may be allocated to alkyl chains (alkaloids) [26]. The peak centred at 1647 cm^{-1} may be due to C = O (anthraquinones) [27]. The peak occurring at 1104 cm^{-1} may be due to the stretching mode of C - O, while the band appearing at 845 cm^{-1}, may be attributed to = C = H alkenes (lignins) [27, 28]. The band at 780 cm^{-1} may be due to the Ce - O - Ce bond [28]. The presence of the

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prominent band at 472 cm^{-1} may serve as confirmation that O - Ce - O nanoparticles have formed [29, 30].

TABLE - 7

Comparison of the results of FTIR analysis with earlier works

S. No.	USED BIOAGENT	IDENTIFIED PHYTOCHEMICAL CONSTITUENTS	REFERENCE
1	*Acacia Concinna Plant*	phenols, aminoacids and flavonoids	Muduli et al., 2023 [26]
2	*Prosopis farcta Fruit*	phenols, alkaloids and flavonoids	Miri et al., 2018 [27]
3	*Alovera gel*	anthraquinones, enzymes, vitamins, amino acids, lignins, monosaccharide, salicylic acids, polysaccharides, saponins and sterols	Priya et al., 2014 [28]
4	*Cucumis sativus* Finch	alkaloids, phenols, aromatics and Saponins	Zaho et al., 2014 [29]
5	*Thermophilic fungus Humicola sp.*	phenols, alkaloids, aromatic rings and proteins	Khan et al., 2013 [30]
6	*Piper betle L.* Leaves	alkaloids, phenols, aromatic compound, saponins, steriods and anthraquinones	**Present Work**

Table - 7 illustrates the comparison of the outcome of the current FTIR investigation with those reported by earlier workers [26 - 30]. From Table - 8, it is quite obvious that phenols and anthraquinones were found to be invariably present in all the three chosen samples namely, Aqueous leaf extract, as - synthesized and biosynthesized CeO_2 nanosamples.

TABLE - 8

Outcome of the present FTIR analysis

Phytochemical Present	Available in		
	Crude *Piper betel L.* leaf extract	Before calcination Sample	After calcination Sample
Alkaloids	-	+	+
Aromatic compound	+	-	-
Phenols	+	+	+

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Anthraquinones	+	+	+
Saponins	-	+	+

(+) indicates Presence; (-) indicates Absence

E. PHYTOCHEMICAL ANALYSIS

Piper betel L. leaf extract as well as CeO_2 nano powder (before and after calcination) were qualitatively evaluated for their phytochemical constituents. Table - 9 represents the outcome of the phytochemical analysis carried out in the present investigation. A close inspection of Table - 9 shows that the leaf extract of *Piper betel L.* consists of Flavonoids, Coumarins, Alkaloids, Glycosides, Carbohydrates, Saponins, Phenols, Starch, Quinones, Steroids, Terpenoids and Tannins. It shows the presence of Coumarins, Carbohydrates, Glycosides, Phenols, Terpenoids, and Steroids in the case of the as - synthesised CeO_2 nanoparticles. In the case of the biosynthesized CeO_2 nanoparticles, it shows that Carbohydrates, Coumarins, Steroids, Glycosides, and Phenols are present. Table - 10 represents the comparison of the results of the qualitative phytochemical analysis, as carried out in the present study, with those reported by earlier workers [31 - 34].

TABLE - 9

Raw Qualitative Phytochemical Analysis of Crude leaf extract, As - synthesized and Biosynthesized CeO_2 nanosamples

S. No.	Phytochemical Present	Aqueous leaf extract of *Piper betel L.*	Bioinspired CeO_2 Nanosample	
			Before Calcination sample	After Calcination sample
1	Acids	-	-	-
2	Alkaloids	+	-	-
3	Anthocyanins and Betacyanins	-	-	-
4	Carbohydrates	+	+	+
5	Cardiac glycosides	-	-	-
6	Coumarins	+	+	+
7	Flavonoids	+	-	-
8	Glycosides	+	+	+
9	Phenols	+	+	+
10	Proteins	-	-	-
11	Quinones	+	-	-

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12	Saponins	+	-	-
13	Starch	+	-	-
14	Steroids	+	+	-
15	Tannins	+	-	-
16	Terpenoids	+	+	+

(+) indicates Presence; (-) indicates Absence

A close scrutiny of Table - 9 reveals the fact that certain phytochemicals were commonly detected in all the three cases. Phytochemical constituents such as Carbohydrates, Coumarins, Glycosides, Phenols and Terpenoids were commonly noticed in all the three samples, chosen for the present investigation.

TABLE - 10
Comparison of the results of phytochemical analysis with earlier works

S. No.	Bioagent	Solvent Used	Identified Phytochemical Constituents	Reference
1	*Piper betel L.* Leaves	Methanolic extract	alkaloids, flavonoids, saponins, steroids, tannins and terpenoids	Murugesan et al., 2020 [31]
2	*Piper betel* plant	Aqueous extract	alkaloids, flavonoids, phenols, anthraquinones, steroids,steroid glycosides, tannins and terpenoids	Venkateswarlu et al., 2014 [32]
3	*Piper betel L.* Leaves	Petroleum ether Extract	flavonoids, phenols, phytosterols, saponins, tannins and terpenoids	Singha et al., 2011 [33]
4	*Piper betel* Inflorescence	Methanolic extract	alkaloids, flavonoids, glycosides, phenols, steroid and tannins	Sushma et al., 2000 [34]
5	*Piper betel L.* Leaves	Aqueous extract	carbohydrates, flavanoids, coumarins, alkaloids, phenols, quinones, glycosides, saponins, starch, steroids, tannins and terpenoids	**Present Work**

This findings pertaining to the phytochemical analysis provide ample evidence that *Piper betel L.* leaves may supply requisite phytochemical which may serve as both reducing and capping agent in the production of CeO_2 NPs. Thus, the outcome of the present investigation, indicating the

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availability of various phytochemicals even in the case of the prepared biogenic CeO_2 NPs, signifies that the identification of the phytochemical constituents may be helpful when looking for biogenic substances and bionutrients that could lead to the discovery of new drugs.

F. GAS CHROMOTOGRAPHY / MASS SPECTRAL ANALYSIS

In order to investigate for the available bioactive compounds, the aqueous extract of *Piper betel L.* leaves, as - synthesized (before calcination) and biosynthesized (after calcination) CeO_2 nanosamples were subjected to GC - MS analysis. The qualitative study was carried out by comparing the retention time and mass obtained for the selected samples with the collective database created by NIST. Table - 11 contains the findings of the qualitative study done on the crude extract of *Piper betel L.*, whereas Fig. 8 displays the gas chromatogram.

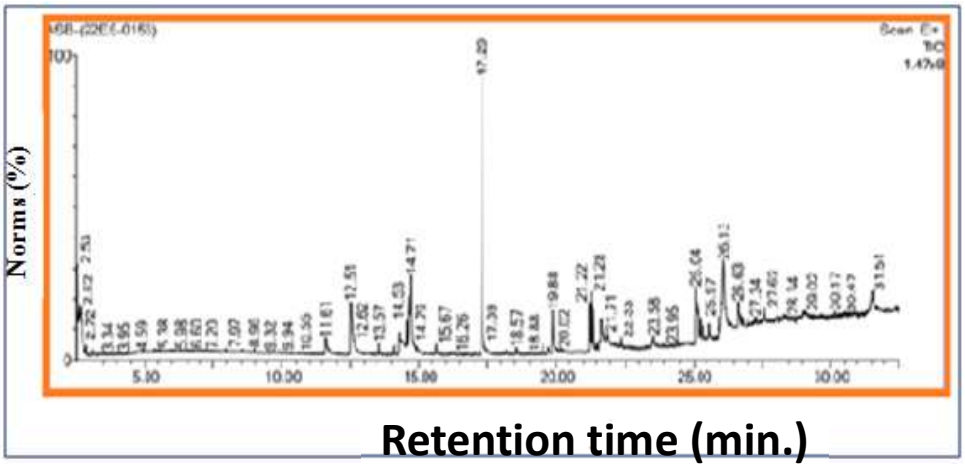

Fig. 8 Gas chromatogram of the crude leaf extract of *Piper betel L.* leaves

TABLE - 11

GC - MS analysis of *Piper betel L.* Crude leaf extract

S. No.	RT VALUE (min.)	Tentative Assignment	NORMS (%)
1	2.528	Silane, Diethyl (Tannins)	19.02
2	2.633	N- Methyltaurine (Acids)	54.78
3	12.512	Phenol, 2- Methoxy-3-(2)	38.15
4	14.288	Aromadendrene (Aromatic ring)	19.51
5	14.623	Spiro Carboxylic acid	19.62
6	14.708	Phenol, Acetate	39.56
7	17.295	2,6- Dibromotoluene (hydrocavicol)	100.00
8	19.881	N- Hexadecanoic acid (Fatty Acids)	19.52
9	21.656	Phytol (flavonoids)	19.35
10	25.043	Benzene, (1-octyl dodecyl) (Phenyl)	19.08
11	25.093	Dodecane,1-Chloro-(Hydroxychavicol)	13.25

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12	26.133	Phthalic acid (Alkaloids)	64.13
13	31.530	Dodecanal (Phenols)	17.82

Table - 11 reveals that a variety of phytochemicals, like tannins, acids, aromatics, carboxylic acids, phenols, hydrocavicols, fatty acids, flavonoids, and alkaloids, are present. Similar observations have been made by earlier workers. Table - 12 indicates the difference between the results of the current GC - MS analysis with those reported by other researchers [35 - 38]. The results we obtained indicate that certain phytochemical components, including phenols, flavonoids, and tannins, are essential for the synthesis of nanoparticles.

TABLE - 12

Comparison of the results of GC - MS analysis with earlier works

S. No.	**BIOAGENT**	**IDENTIFIED PHYTOCHEMICAL CONSTITUENTS**	**REFERENCE**
1	*Piper betel L.* Leaves	flavonoids, phenols and carbohydrates	Madhumita et al., 2019 [35]
2	*Piper betel* plant	fatty acids, phenols, flavonoids and tannins	Annegowda et al., 2013 [36]
3	*Piper betel L.* Leaves	flavonoids, phenols, acids, phytol and carbohydrates	Dwivedi et al., 2010 [37]
4	*Piper betel L.* Leaves	flavonoids, phenols, phytosterols, Saponins and tannins	Arambewela et al., 2005 [38]
5	*Piper betel L.* Leaves	alkaloids, acids, flavonoids,phenols, aminoacids, saponins, and hydrocavicol	**Present Work**

Fig. 9 illustrates the Gas chromatogram recorded for as - synthesized (before calcination) CeO_2 nanoparticles while Table - 13 represents the tentative assignment of the observed peaks.

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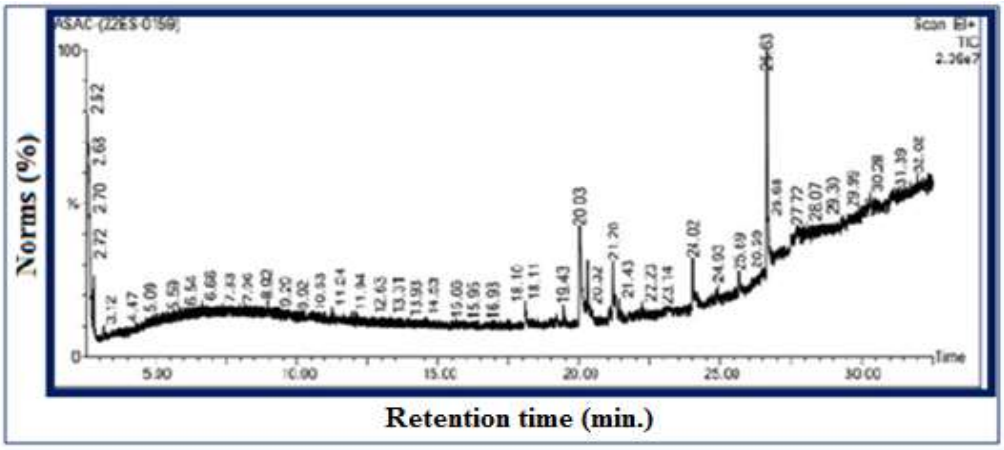

Fig. 9 Gas Chromatogram of as - synthesized (before calcination) CeO_2 nanosample

TABLE - 13

GC - MS analysis of as - synthesized (before calcination) CeO_2 nanosample

S. No.	RT VALUE (min.)	Tentative Assignment	NORMS (%)
1	2.623	N- Methyltaurine (Acids)	100.00
2	2.829	Hexopyranose (Flavonoids)	4.46
3	18.110	2- Undecanone 2,4 – Dintrophenylhydrazone (Carbohydrates)	3.49
4	20.026	4- Undecene, 10- methyl-, (Carbohydrates)	21.68
5	20.271	Trimethyl tetradecanol (Alkaloids)	6.38
6	21.196	Methyl ester (Acid)	5.82
7	21.256	Aminonenadecane (Aminoacid)	4.56
8	24.022	Trimethyl tetradecanol (Alkaloids)	4.58
9	25.688	1- octadecanamine, N-Methyl- (Terpinoids)	3.04
10	26.633	propenoic acid (Phenols)	30.48

From Table - 13 it is clear that the as - synthesized (before calcination) CeO_2 nanosample contains bioactive compounds such as Acids, Flavonoids, Carbohydrates, Alkaloids, Terpenoids, and Phenols. Fig. 10 shows the Gas chromatogram recorded for the biosynthesized (after calcination) CeO_2 nanoparticles while Table - 14 represents the results of the qualitative analysis made on it.

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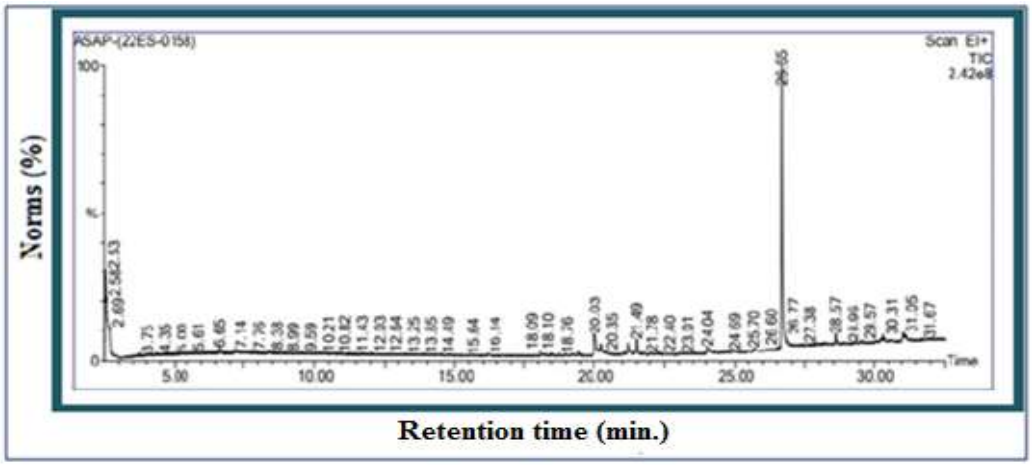

Fig. 10 Gas chromatogram of biosynthesized (after calcination) CeO_2 nanoparticles

TABLE - 14

GC - MS analysis of synthesized (After calcination) CeO_2 nanopowder

S. No.	RT VALUE (min.)	Tentative Assignment	NORMS (%)
1	2.533	Silane, Diethyl (Tannins)	86.96
2	20.030	5- tridecene, (Flavonoids)	15.17
3	21.491	2,6- Dibromotoluene (Hydrocavicol)	4.92
4	26.653	Heptane,3-(Bromomethyl) (Flavonoids)	100.00
5	28.569	Dodecanal (Phenols)	4.96

From Table - 14, it is quite obvious that phytochemicals such as Tannins, Flavonoids, Hydrocavicol and Phenols, available in the case of the crude leaf extract, were also present in biosynthesized CeO_2 nanosamples, even after calcination.

TABLE - 15

Outcome of the present GC - MS analysis

Phytochemical Identified	Available in		
	Crude *Piper betel L.* leaf extract	Before calcination Sample	After calcination Sample
Alkaloids	+	+	-
Carbohydrates	-	+	-
Acids	+	+	-
Flavonoids	+	+	+

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Hydrocavicol	+	-	+
Phenols	+	+	+
Tannins	+	-	+
Terpenoids	-	+	-

(+) indicates Presence; (-) indicates Absence

From Table - 15, it is crystal clear that Phenols and Flavonoids were noticed to be invariably present in all the three chosen samples namely, aqueous leaf extract, as - synthesized (before calcination) and biosynthesized (after calcination) CeO_2 nanosamples. This striking finding reveals the crucial role of phytochemicals in the development of *Piper betel L.* mediated CeO_2 NPs and hence, it may be expected that the *Piper betel L.* inspired CeO_2 nanoparticles may exhibit an excellent antimicrobial and other biogenic activities.

G. ^{1}HNMR SPECTRAL ANALYSIS

Fig. 11 and Table - 16 (a) represent the spectrum and the results of ^{1}HNMR spectral analysis of crude extract of *Piper betel L.*, leaves respectively.

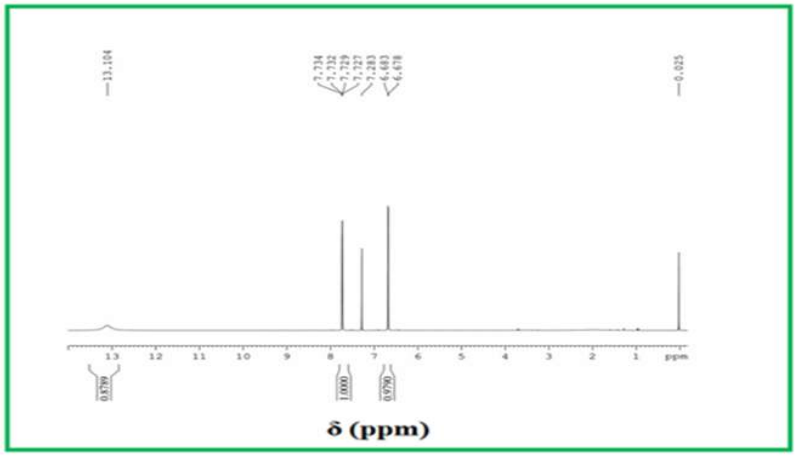

Fig. 11 ^{1}HNMR spectrum of Aqueous leaf extract of *Piper betel L.*

A close scrutiny of Table - 16 (a) reveals the following valid informations: The ^{1}HNMR peak at 0.025 ppm may be attributed to the presence of hydroxyl group (Panda et al., 2003). According to Gharpure et al., (2022), the ^{1}HNMR signals noticed in the region 6.678 - 6.683 ppm may be assigned to aromatic rings.

TABLE - 16

Tentative Assignment for the ^{1}HNMR spectrum for (a) Aqueous leaf extract of *Piper betel L.* (b) as-prepared and c) bioprepared CeO_2 nanosample

(a) Crude leaf extract of *Piper betel L.*			
S. No.	**Peak Value (ppm)**	**Tentative Assignment**	**Phytochemical name**
1	0.025	Hdroxyl group	Phenols
2	6.678 - 6.683	aromatic ring	Phenols
3	7.283	- C_6H_5 group	Phenols
4	7.727 - 7.734	- C_6H_5 group	Phenols
5	13.104	C_{15} $H_{12}O_5$	Flavonoids
(b) before calcination (as - synthesized) sample			
S. No.	**Peak Value (ppm)**	**Tentative Assignment**	**Phytochemical name**
1	1.780	aliphalitic group	Phenols
2	2.511	- C_6H_5 group	Phenols
(C) after calcination (biosynthesized CeO_2) sample			
S. No.	**Peak Value (ppm)**	**Tentative Assignment**	**Phytochemical name**
1.	4.532 - 4.611	polar group of phenols	Phenols

The appearance of phenols in the analyzed samples may be approved from the occurrence of three types of down - field peaks at 7.283, 7.727 and 7.734 ppm (Mozumder et al., 2020). The peak at 13.04 ppm [Evangelos et al., 2006; Exarchou et al., 2002] may be an indication of the presence of flavonoids.

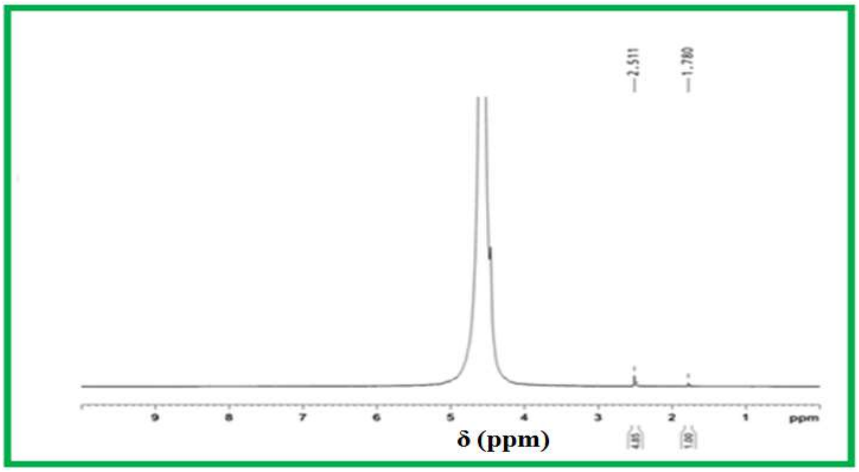

Fig. 12 ^{1}HNMR spectrum of as - prepared (CeO_2) nanosample

Fig. 12 illustrates the ^{1}HNMR spectrum recorded for the as - synthesized (before calcination) CeO_2 nanoparticles. The tentative assignment of various NMR peaks occurring in the case of as - synthesized CeO_2 nanoparticles, is being represented as Table - 16 (b) and the following observations have been made:

The peak at 1.780 ppm may be due to the presence of aliphatic group (Lopez - Martinez et al., 2015). While the signal centered at 2.511 ppm may be due to the phenolic group (Osman et al., 2021).

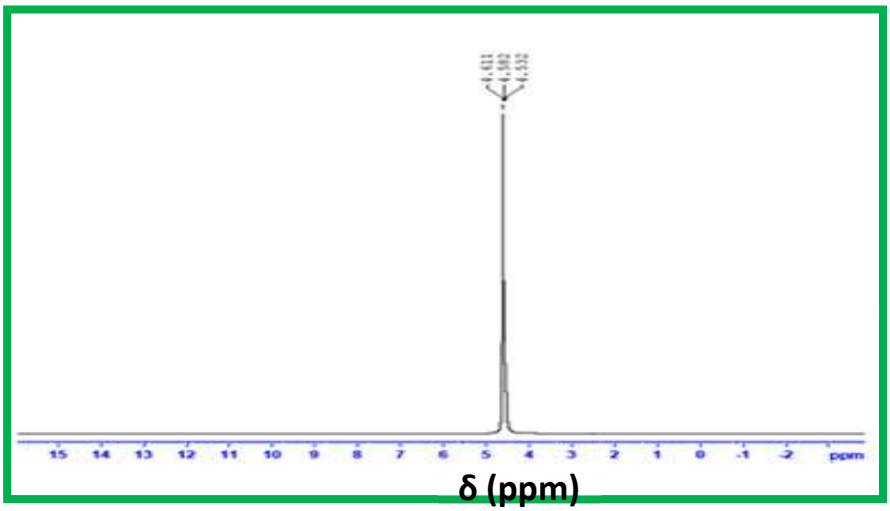

Fig. 13 ^{1}HNMR spectrum of biosynthesized (CeO_2) nanoparticle

Fig. 13 represents the ^{1}HNMR spectrum for biosynthesized CeO_2 nanoparticles while Table - 16 (c) represents the tentative assignment done for the proton NMR peaks occurring in the spectrum. The proton NMR peaks positioned at 4.532 - 4.611 ppm, as detected in the present study, may be due to the polar group of phenols (Gharpure et al., 2022)

Table - 17 illustrates the comparison of the results of the present work with those reported earlier [39 - 45]

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TABLE - 17

Comparison of the results of ^{1} HNMR analysis with earlier works

S. No.	BIOAGENT	IDENTIFIED PHYTOCHEMICAL CONSTITUENTS	REFERENCE
1	*Cocos nucifera* Extract	Hydroxyl group, aromatic group, alkyl group and phenols	Gharpure et al., 2022 [39]
2	*Piper betel L.* Leaves	Phenols, hydroxyl group and Flavonoids	Osman et al., 2021 [40]
3	*Camellia sinensis* var. Plant	Phenols, fatty acids, sucrose and glucose	Mozumder et al., 2020 [41]
4	*Manguifera indica, Carica papaya* Fruit	Phenols, carboxylic acid, aromatics and hydroxyl group	Lopez - Martinez et al., 2015 [42]
5	*Hypericum perforatum* Flower	Phenols, carbonyl group, hydroxyl group and flavonoids	Evangelos et al., 2006 [43]
6	*Piper betel L. seeds*	hydroxyl group, phenyl group and flavonids	Panda et al., 2003 [44]
7	*Oregano vulgare L.* Leaves	Phenols, Flavonoids and hydroxyl group	Exarchou et al., 2002 [45]
8	*Piper betel L.* Leaves	Phenols, aromatics and Flavonoids	**Present Work**

TABLE - 18

Outcome of the present ^{1}HNMR analysis

Identified Phytochemical	Available in		
	Crude *Piper betel L.* leaf extract	Before calcination Sample	After calcination Sample
Phenols	+	+	+
Aromatics	+	-	-
Flavonoids	+	-	-

(+) indicates Presence; (-) indicates Absence

From Table - 18 it is quite obvious that phenols occur in all the three chosen samples namely, crude leaf extract, as - synthesized (before calcination) and biosynthesized (after

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calcination). This interesting observation confirms the inevitable role played by phenols in the formation of *Piper betel. L* induced CeO_2 nanoparticles.

CONCLUSION

In the present work, the aqueous extract of *Piper betel L.* was used to biologically synthesized CeO_2 nanoparticles and calcined at 850 ºC. From the XRD and SEM analyses, the synthesized nanosample was found to be cubic crystal, in nature, with average 'D' value being 49 nm. The outcome of the EDAX studies confirmed the purity of the sample while FTIR spectral analysis ascertained the formation of O - Ce - O bond in the case of *Piper betel L.* induced CeO_2 nanoparticles. The qualitative phytochemical analysis showed the availability of various phytochemicals in all the three chosen samples, namely aqueous leaf extract, as - synthesized (before calcination) and biosynthesized (after calcination) CeO_2 nanosamples. The results of FTIR, GC - MS and ^{1}HNMR spectral studies clearly portrayed the presence of phenols in all the three samples and thereby ascertained the pivotal role of phenols in the formation of CeO_2 nanoparticles. Since CeO_2 nanosamples exhibited good phytochemical activity, it may be concluded that the *Piper betel L.* induced CeO_2 nanoparticles may serve as efficient bioagent for various biomedical applications.

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DECLARATION

The author attests that they have no financial conflicts of interest with regard to this study.

DISCLOSURE STATEMENT

The author did not disclose any potential conflicts of interest.

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