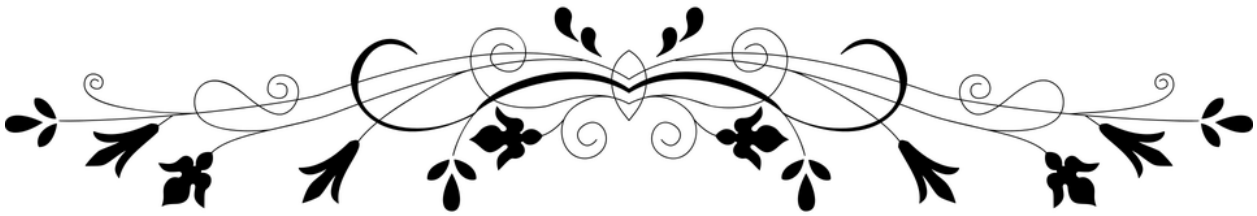

CHAPTER 2

Literature Review

CHAPTER 2

2.1 Literatures related to tribasic copper chloride (Atacamite)

Copper (II) hydroxy chloride [$Cu_2(OH)_3Cl$] is a natural copper hydroxide based biomineral generated via rich chemistry of copper ion and currently explored in the field of analyte sensing [120], antimicrobial usage [121], hydrogen storage [122] environmental pollutant degradation [123, 124], and magnetism related applications [125].

The tribasic copper chloride [$Cu_2(OH)_3Cl$] is reported to exist in four polymorphic forms, viz. Atacamite, Botallackite, Paratacamite, and Clinoatacamite [117, 126]. They act as green color pigments and are recovered extensively as natural minerals from the copper mining process [127]. Very limited number of researchers given their effort towards synthesizing various polymorphic forms of tribasic copper chloride in laboratory-scale over the decade. Moreover, most of the reported researches till now involved mainly two mechanisms (i) aqueous phase reaction of copper metal under basic medium (ii) direct precipitation from copper chloride salt. Based on these mechanisms, literatures related to the laboratory-scale synthesis for copper hydroxy chloride biominerals are reviewed here briefly (Table 2.1). Botallackite nanoplates have been synthesized via reduction in the alkaline medium [128] as reported by yang et al. These nanoplates have been used as precursor material towards the development of CuO nano rods and sheets. Their work demonstrates an aqueous phase synthesis technique, in which vigorous mixing of two or more types of copper salts and subsequent washing need to occur. Similarly, Chen et al. [129] have developed the solid and hollow flower-like architecture of botallackite nanostructure with an average diameter of 500 nm. The synthesis is carried out hydrothermally under highly alkaline conditions (maintained

by aqueous ammonia) using copper chloride salt as precursor material and cetyltrimethylammonium bromide (CTAB) as chelating ligand. In another study, Zheng et al. [130, 131] have reported the preparation of microcrystals of botallackite from cupric formate tetrahydrate and potassium chloride at room temperature. Furthermore, via extensive magnetic characterizations (magnetic susceptibility and muon spin rotation/ relaxation) the correlation for antiferromagnetic transitions from bulk to micro-scale have developed. The synthesis is carried out in an aqueous phase by adjusting temperature and solution concentration. Similarly, Zhao et al. [132] have also studied regarding compression behavior of botallackite nano-flakes prepared under hydrothermal conditions. The template-free one-step hydrothermal synthesis strategy has led to production of phase pure nanoflakes with thickness of 30-40 nm and lateral dimension ranging up to 200 nm. The structural and mechanical stability of prepared botallackite nanoflakes, have investigated by the synchrotron radiation angle-dispersive X-ray diffraction (ADXRD) technique. However, the hydrothermal technique adopted in the study has involved elevated temperature (~150 ^{o}C) and long reaction time duration. The research has also been carried out to develop aggregated monodispersed platelets type morphology of Paratacamite polymorph [122]. Synthesis has carried out by heating a mixture of $CuCl_2 \cdot 2H_2O$, urea, 1-octanesulphonic acid sodium salt, and water at 100 ^{o}C for a long time. Resulting spherical aggregates have been effectively used in hydrogen storage applications. In another study, Liu et al. [125] have carried out the successful synthesis of Paratacamite microcrystals by facile hydrothermal method and subsequent doping of Ni element in the crystal structure. Effective development of bare and Ni-doped Paratacamite nanocrystals are the result of very long duration (~20 h) synthesis strategy at an elevated temperature of 180 ^{o}C. The resulting Paratacamite microcrystals have

been investigated for their possible application in the field of photocatalysis. Similarly, Padmapriya et al. [133] have investigated regarding hydrogen storage potential of Paratacamite doped polypyrrole by wet chemical method using $CuCl_2$ as an oxidant. Briefly saying chemical oxidative polymerization of pyrrole monomer has carried out under acidic medium and subsequent electrochemical characterizations indicated good hydrogen storage capability. Further copper hydroxy chloride-polypyrrole nanocomposite has been prepared by a single-step in-situ chemical oxidation step. This has been reported by Kasisomayajula et al. [134] and in their study they have described regarding the formation rate of above mentioned composite and variation of rate of reactions under controlled conditions. They have also investigated regarding electrical conductivity and magnetic properties of polypyrrole-copper hydroxy chloride nanocomposites. Clinoatacamite another polymorph of tribasic copper chloride, successfully synthesized in laboratory scale by pH control [135]. The one-pot synthesis strategy has involved pH control of $CuCl_2$ precursor solution by employing a buffer "2-(N-morpholino) ethane sulfonic acid (MES)". However, the process required extensive control of reaction parameters and involved rigorous filtration steps.

Among the above-described polymorphs of tribasic copper chloride "Atacamite" has been notably studied due to its distinct physicochemical and crystallographic properties [136, 137] in addition to frustrated magnetic behavior [138]. It was initially discovered from the jaws of marine carnivorous worm Glycera dibranchiata [139]. Extensively mined at "Atacama" dessert in Chile, thus named after this place [126]. Wolf et al. [140] have carried out polyol-mediated synthesis of crystalline Atacamite with size close to 50 nm. The synthesized Atacamite nanomaterial have been used as a precursor material for preparation of conducting thin films by depositing on the silicon wafer/glass/paper substrates, following a chemical

reduction route. Furthermore Zhu et al. [141] have synthesized Atacamite nanoribbons with 50-60 nm in diameter, and several microns in length. They have adopted a template-free approach involving bi-hydrolyzation of $(NH_4)_2CO_3$ precursor at room temperature. The prepared Atacamite nanoribbons have been employed as a sacrificial template for the development of CuO nanostructure on heat treatment under elevated pressure. There is also a report regarding the bi-pyramidal architecture of Atacamite microparticles prepared by homogeneous precipitation of copper chloride salt solution in the presence of urea [142]. These microparticles afterward have been used for fabrication of $Cu(OH)_2$ and CuO structure upon NaOH addition and high-temperature calcination, respectively. Furthermore, octahedral Atacamite microcrystals have been successfully synthesized on a laboratory scale by Zhang et al. [137] for possible applicability in the field of optical device fabrication. In this method, a facile hydrothermal route has adopted for synthesis of Atacamite microcrystals using copper salt and aqueous solution of urea. But reaction is carried over a long time duration.

Table 2.1 Brief description of Atacamite and its other similar polymorphs synthesized in laboratory scale by various group of researchers.

Sl. No.	Polymorph of tribasic copper chloride	Synthesis route	Morphology produced	Application	Reference
1	Botallackite	Aqueous phase synthesis	Nanoplates	Produced CuO nanorods	[128]
2	Botallackite	Hydrothermal	Solid/hollow nano-flower with diameter upto 500 nm	Physico-chemical characterization	[129]
3	Botallackite	Aqueous phase synthesis	Microcrystals	Magnetic characterization	[130, 131]
4	Botallackite	Hydrothermal	Nano-flakes	Physical characterization	[132]
5	Paratacamite	Long duration heating of aqueous phase mixture	monodispersed platelets	Hydrogen storage	[122]
6	Paratacamite	Hydrothermal	Microcrystals	Photocatalysis	[125]
7	Paratacamite	Wet chemical oxidation	Polymers	Hydrogen storage	[133]
8	Clinoatacamite	One pot solution phase synthesis by pH control	Nano-whiskers	Physico-chemical characterization	[135]

9	Atacamite	Polyol mediated synthesis	Spherical	Conducting thin film	[140]
10	Atacamite	Template free synthesis	Nano-ribbons	Formation of CuO nanostructure	[141]
11	Atacamite	Precipitation of salt solution	Bi-pyramidal	Formation of CuO and $Cu(OH)_2$	[142]
12	Atacamite	Long duration hydrothermal reaction	Octahedral microcrystals	Optical device	[137]

Though Atacamite originated from the biological route, the application of this important semiconducting biomineral in the biological field (especially in cancer theranostics) is very much scarce. Few lab-scale synthetic routes reported above have demonstrated the formation of Atacamite nano/microstructures, e.g., octahedral [137] pyramidal microcrystal [142], flower-like architecture [129], nanoribbons [141], and nanoplatelets [122], etc. From usability point of view Atacamite is also employed as a raw material to produce Cu [140], CuO [128], and $Cu(OH)_2$ conducting ink, tape, and catalyst. Further they have also been used in hydrogen storage [122] and optical applications [125]. Unfortunately, studies concerning size reduction of Atacamite crystals to nano/sub-nanoscale dimensions (e.g. nanoclusters/dots) and the corresponding effect on light absorption, fluorescence, and the semiconducting band structure are remain unexplored. Further the possible modification in the frustrated magnetic behavior of Atacamite crystals within nanoscale dimensions has never been explored. Therefore in view of possible applicability in theranostics as well as other diverse physicochemical sectors the proposed modulation in the magnetic and photo-physical property of Atacamite nanomaterials need to be essentially investigated.

2.2 Exciting properties of Copper oxide Nanomaterials

Similar to Atacamite, copper oxide is also another important biomineral [143] widely developed in the lab scale. It is an important class of p-type semiconductor material known to exist in two phases CuO (1.8–2.5 eV) and Cu_2O (1.2–2.0 eV) [144]. Nanomaterials of copper oxide could be a suitable candidate for cancer theranostics purposes besides demonstrating improved photo-physical, band-edge transitions, and paramagnetic characteristics. But their applicability is more or less limited to the toxic impact on the cancerous cell (Table 2.2) by employing them as a chemotherapeutic drug. Nevertheless,

prevailing attributes like “surface plasmon resonance”, “semiconducting band transition,” and “controlled release of covalent bound electrons” of copper oxide nanomaterials have been limitedly investigated in advanced cancer treatment techniques like “photothermal therapy.” Further, via quantum confinement effect and direct bandgap nature, the nanostructures of these semiconducting metal oxides are known to exhibit interesting electrochemical activity that has their own potency in electrochemical biosensing and electrochemical energy storage, etc. Combined cancer therapeutic and electrochemical functionality of ultra-small copper oxide-based nanomaterials is very interesting to watch out.

Table 2.2 Cytotoxicity effect of copper oxide nanomaterials to cancer cell lines.

Sl no.	Leaf Extract used for Copper oxide nanoparticle synthesis	Morphology & Size	Biomedical application	Reference
1	*O. cochinchinense*	Agglomerated clusters (2 µm)	Cytotoxic effect on human colon cancer cell lines HCT-116	[145]
2	*Olea europaea*	Spherical (~ 20 nm)	Cytotoxic activity for AMJ-13 and SKOV-3 cancer cell lines	[146]
3	*Acalypha Indica*	Aggregated clusters	*In Vitro* toxic effect on HCT-116 cells	[147]
4	*Ficus religiosa*	Irregular morphology	Anticancer activity against human AS49 lung cancer cells	[148]
5	*Camellia Sinensis*	Spherical (~ 30 nm)	Potential toxicity against MCF-7 (breast cancer cells)	[149]
6	*Coleus aromaticus*	Spherical (17-40 nm)	Effective miRNA-29b delivery to A549 lung cancer cells thus improving therapeutic efficacy	[150]
7	*Azadirachata Indica*	Spherical (~36 nm)	Apoptosis of MCF-7 AND Hela cells mediated via TNF-α and caspases signaling pathway	[151]
8	*Duchesnea indica*	Spherical (~ 80 nm)	Cytotoxic to A-498 kidney tumor cells	[152]

2.3 Multifunctional Copper oxide/hydroxide based Biominerals

Overall lattice symmetry alteration, modified electrochemistry, band gap induced conductivity, chemical activity, and magnetism based properties lead to multiple functionalities of semiconducting copper oxide and hydroxide based nanomaterials. This is also the main objective of the current study. By keeping in mind above mentioned advantages, an extensive investigation has been carried out for studying multifunctional properties of previously reported copper oxide/hydroxide based biominerals (Table 2.3). This not only explain the drawbacks associated with the previous reported researches but also open-up an exciting pathway for obtaining the best possible results in diverse biological and physicochemical sectors via a cost-effective manner. The majority of highlighted researches (Table 2.3) are based on the multimodal usability of copper oxide in medicine, biotechnology, catalysis, etc. But Atacamite nanomaterials have been limitedly researched. Hence to have a parallel comparison, some other copper hydroxide based biominerals have been investigated for their multiple functionalities.

Table 2.3 Reported literatures related to multifunctional applications of copper metal-based semiconducting nanomaterials.

Sl no.	Copper oxide/ hydroxide based nanosytem	Morphology & Size	Synthesis Route	Multifold applications	Reference
1	CuO + Cu_2O	Irregular (~28 nm)	*Achillea millefolium* leaf extract	Antibacetrial, antifungal, catalytic/photo-catalytic	[153]
2	CuO	Spherical (~18 nm)	*Malus domestica* leaf extract	Antibacterial, antioxidant, DNA damage	[154]
3	Copper oxide	Clusters (~5 nm)	*Abies spectabilis* aerial part extract	Antinociceptive and anti-inflamatory potential	[155]
4	CuO	Irregular (20-60 nm)	*Allium sativum* extract	Antimicrobial, antioxidant, antilarvicidal	[156]
5	Copper oxide	Spherical (~33.47 nm)	*Beta vulgaris L* extract	Antibacterial and anticancer activity	[157]
6	CuO	Spherical (5-22 nm)	*T. terrestris* fruit extract	Antibacetrial and cytotoxicity to cancer cells	[158]
7	CuO	Spherical (5-13 nm)	*S. alternifolium* stem bark extract	Antimicrobial and anticancerous	[159]

8	CuO	Cluster (~12 nm)	*Azadirachta indica, Hibiscus rosa-sinensis, Murraya koenigii, Moringa oleifera* and *Tamarindus indica* extract	Antioxidant and anticancer activity	[160]
9	CuO	Voids and pores	*Tinosporia cordifolia* leaf extract	Photocatalytic, antioxidant, antibacetrial	[161]
10	CuO	Cluster (~ 20-35 nm)	*C. sebestena* flower extract	Chemical catalysis, photocatalyst, antibacterial	[162]
11	CuO	Spherical, cuboid, oval (~13 nm)	*Eucalyptus globulus* leaf extract	Anticancerous and antifungal	[163]
12	CuO	Irregular	*Verbascum thapsus* leaf extract	Photocatalysis Antibacterial effect	[164]
13	CuO	Irregular	*Abutilon indicum* leaf extract	Antimicrobial, antioxidant & photocatalytic activity	[165]
14	CuO and Ag/CuO	Quasi sphere (3.56 nm) and rod (2.8 nm)	*Sida Rhombifolia* leaf extract	Antibacterial activity, chemical catalysis & adsorption	[166]
15	CuO	Nanosheet (w: 103.7 nm) & Nanorod (27.3 nm)	*Terminalia catappa* leaf extract	Environmental catalysis & colorimetric sensing	[167]
16	Copper oxide	Spherical (26-30 nm)	*Acalypha indica* leaf extract	Antimicrobial & anticancerous	[168]
17	CuO	Spherical aggregate (20-40 nm)	*Solanum lycopersicum* leaf extract	Antibacterial, photocatalytic dye degradation	[169]

18	CuO	Spherical (~10-50 nm)	*Pterolobium hexapetalum* leaf extract	Antibacterial, photocatalytic & anticancerous effect	[170]
19	Copper hydroxide	Nanowire 500 nm length& ~10 nm dia.	Pistachio leaf extract	Physico-chemical characterization	[171]
20	$Cu(OH)_2$	Irregular (~19.4 nm)	*Nymphaea Rubra* leaf extract	Antibacterial Activity	[172]
21	$Cu(OH)_2$	Colloidal nanohybrid	Chemical reduction & polyelectrolyte stabilized	Optical response	[46]
22	$Cu(OH)_2$	Nanocage (~256 nm)	Chemical reduction	Structural property	[173]
23	$Cu(OH)_2$	Spherical nanoparticle (~230 nm)	Chemical reduction-Glycine stabilized	Biocide, antimicrobial	[174]
24	$Cu(OH)_2$	Branched Nanocage (length ~74 nm and dia ~221 nm)	Chemical reduction	Copper oxide formation	[175]
25	$Cu_2(OH)PO_4$	Quantum dots (~ 4 nm)	Chemical synthesis	Photoaquostic imaging Photothermal therapy Photodynamic therapy	[176]

2.4 Photo-Physical applications of Tm^{3+} doped Upconverting phosphors

The insufficient heating of deep tissues, and their achieved thermal resistance after initial exposure due to low heat generation rate is responsible for limited efficacy of photothermal therapy in cancer treatment [19]. Further there are chances of biological interference during cancer diagnosis and bio-analytical processes, while using fluorescence based systems also. Keeping in view the above-discussed constraints better results would obviously be obtained by using trivalent lanthanide ion-doped Upconverting phosphors as earlier stressed. Trivalent thulium (Tm^{3+}) doped Upconverting phosphors have attracted wide attention in the scientific community due to their strong UV as well as blue light emission and inertness in the surrounding environment [177].

Nevertheless, the Tm^{3+} and Yb^{3+} co-doped $NaYF_4$ Upconverting systems could be better for intense luminescence intensity and heat generation capability.

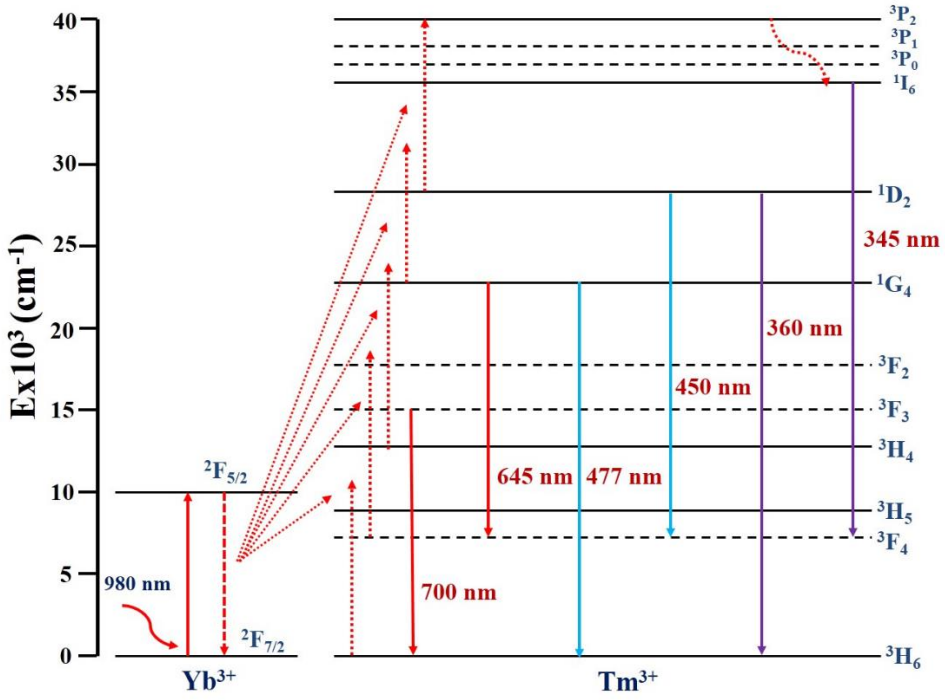

Figure 2.1 Energy level diagram representing luminescence phenomena in Tm^{3+} doped Upconverting phosphors.

Generally, Tm^{3+} doped Upconverting phosphors due to their metastable electronic configuration (Fig. 2.1) can emit characteristic luminescent signal in the UV and blue light region those are used in several optical functions [177]. Also, these specific Upconversion emissions have suitable applicability in certain chemical or biological processes. The violet light can be used to excite some typical family of posing protein for smooth functioning of light-gated ion channels in nerve cells and photochemical catalysis. Blue Upconverting nanocrystals are used for multicolor emitting fluorescent bio labels. This can also be employed in several exciting photosensitizers for cancer diagnostic purposes as well as photodynamic therapy. Generally three or four photons are involved in the Upconversion process of Tm^{3+} based nanosystems, and the blue or UV emission is comparatively low in intensity. Hence residual energy is recovered through the heating effect catalyzed by non-radiative transitions. Therefore Tm^{3+} doped Upconverting phsophors can be employed as probable photothermal agent [178]. Similarly, the autofluorescence issue in the blue wavelength region for biological tissues can be compensated through intense blue color emission from thulium Upconverting nanoparticles and their high signal-to-noise ratio.

A number of researches have been carried out for the development of Tm3+ doped Upconverting phosphors for various practical usage. However in majority of these reported works, has utilised highly efficient NaYF4 as a host lattice during synthesis of UCP. Nonethless, for a brief overview similar types of work based on Tm3+ doped Upconverting phosphrs are reviewed below. Upconversion $NaYF_4$: Yb, (8 % Tm) co-doped system have been developed by Kostyuk et al. [179] for biological labeling of human breast adenocarcinoma cells SK-BR-3. They reported that polyethyleneimine (PEI) is coated on the surface of Upconverting phosphors to impart colloidal stability in an aqueous solution. To

modify the surface of thulium-doped $NaYF_4$ nanophosphor with PEI, nitrosonium tetrafluoroborate ($NOBF_4$) has been used to displace oleate groups via a ligand exchange reaction. The bio-imaging contrast of 8 % Tm doped Upconverting nanophosphors (UCNP) taken up by human breast adenocarcinoma cells SK-BR-3 is achieved by suppressing the background signal using (i) bandpass spectral filter time-correlated single-photon counting system.

In another study, the core-shell $NaYb_{0.99}F_4:Tm_{0.01}@NaYF_4$ Upconverting phosphors with intense UV emission on NIR irradiation have synthesized by Jarosz-Duda et al. [180] for photocatalysis application. NIR absorption is reported to be maximized by increasing Yb^{3+} doping concentration and surface protection by $NaYF_4$ shell-reduced luminesce quenching. The twelve-hour duration solvothermal reaction has produced Upconverting phosphors with ~1.5 µm in size and resulted in intense UV emission to improve the photocatalytic efficacy of associated TiO_2 particles.

A mathematical model is proposed by Bagheri et al. [181] using multivariable statistical analysis and has been reported that the pivotal mechanism to optimize both NIR and blue light emission of Tm-Yb co-doped $NaYF_4$ Upconverting system is dopant ion balance. In their research optimization of the concentration of both ytterbium (Yb^{3+}) sensitizer and thulium (Tm^{3+}) activator in a controlled manner resulting in minimum cross-relaxation between the dopant atoms and energy transfer process could be maximized. The entire aqueous phase reaction was mediated by using toxic, expensive solvents like oleic acid and 1-octadecene. The reaction has been carried out at elevated temperature (~ 300 ^oC) under an inert atmosphere and monodispersed particles with a wide size range (15-150 nm) are generated. Furthermore, Damasco et al. [182] have synthesized monodispersed and size-

tunable Tm^{3+}/Gd^{3+}-Doped hexagonal $NaYbF_4$ nanoparticles in another research. Upon Gd^{3+} doping on controlled manner the cubic phase of 0.5 % doped $NaYbF_4$ irregular shaped particle has been converted into hexagonal phase with monodispersed spherical morphology. The epitaxially grown ~2 nm $NaYF_4$ shell on $NaYbF_4$: 30 % Gd^{3+}, 0.5% Tm^{3+} core, improved the Upconversion emission intensity by 350 times when impinged with 800 nm laser. *In Vivo* NIR-to-NIR imaging has been carried out by effective coating of phospholipid-polyethylene glycol (DSPE-PEG) on prepared nanostructure and dispersed in phosphate buffer saline. The major problem associated with this reported research that, there is extensive use of toxic 1-octadecene and oleic acid during synthesis. Also, reaction required sophisticated mechanisms like the use of argon gas environment and high heating temperature, etc.

Further, Liu et al. [183] have synthesized $NaYF_4$:Yb//Tm@$NaYF_4$ core-shell Upconversion nanoparticle with two-photon NIR emission intensity when excited with low excitation power density. Upon silica encapsulation of prepared core-shell nanoparticle, the *in Vivo* biocompatibility and emission stability is improved significantly. The high signal-to-noise ratio of the prepared core-shell nanoparticles has ascertained from the improved spatial resolution of capillaries and vessels present in a mouse brain at a low power density (~ 25 mW/cm^2).

The variation of dopant concentration on blue Upconversion emission of Tm^{3+} doped ZrO_2 nanocrystals under different excitation wavelengths has been investigated by Patra et al. [184, 185]. The emission tuning of the prepared Upconversion system is the function of "excitation wavelength", and become red-shifted when impinged with radiation of low energy. Tm^{3+} doping in ZrO_2 host lattice has been carried out by complex sol-gel technique,

and pump power investigation revealed that the entire Upconversion process mediated through a two-photon absorption mechanism.

In another study, Pominova et al. [186] have put forward a concept of maximizing the NIR-NIR Upconversion efficiency of Yb^{3+}–Tm^{3+} co-doped hexagonal $NaGdF_4$ host lattice through control of doping ion concentration. A theoretical model has been proposed according to which upon increasing Yb^{3+} concentration up to 80 % from 30 %, the Upconversion emission intensity could be increased by ~10 times. However, optimization of the doping process to obtain a single-phase $NaGdF_4$ structure is achieved by conducting the experiments at 320 ^oC.

Further, Zhan et al. [187] have synthesized Tm^{3+} doped $NaYbF_4$ Upconversion nanophosphors for *in Vitro* and *in Vivo* bioimaging applications without the use of overheating irradiation. For minimizing the possible absorption of 980 nm irradiation by biological specimen and overheating problem during the imaging process, prepared nanomaterials are irradiated with 915 nm light. However, the adopted technique for the preparation of thulium-doped $NaYbF_4$ nanomaterial is a modified co-thermolysis process involving an argon atmosphere and high reaction temperature (~ 320 ^oC). Also, the reagents used are toxic and expensive, such as oleic acid and 1-octadecene. Further the efffective *in Vitro* bioimaging of Hela cancer cell lines with prepared nanophosphors are carried out by conjugation with the specific antibody. Whereas for *in Vivo* bioimaging in the mouse model, these are coated with DSPE-mPEG-5000 molecules.

In their study, Lahtinen et al. [188] have modified the surface chemistry conditions to develop thulium-doped Upconverting nanophosphors previously existed in slightly aggregated stage. The luminescent property of prepared Upconverting nanophosphor is

sufficient enough to be identified within auto-fluorescent plasma samples. The prepared nanophosphors are also shown to have improved performance in an Upconversion cross-correlation spectroscopy-based immunoassay for thyroid-stimulating hormones. Advanced surface chemistry improved the surface coating of prepared nanophosphors such that their aggregation could be minimized in plasma samples. Interestingly with an increase in thulium doping, the blue light emission improved significantly, when irradiated with high excitation power density thereby limiting the long-duration luminescent decay time. This has improved the probability of ascertaining the blue light radiation associated with Upconverting nanophosphors during the use in the immunoassay process. But sophisticated reaction mechanism, toxic solvents, and complex ligand exchange process related to surface modification is major drawback for this study.

In another study, Peng et al. [189] have studied the emission characteristics of Tm^{3+} ion doped Upconverting nanophosphors under NIR-II laser excitation. Their research produced NaYF4:Yb^{3+}, Tm^{3+}core nanoparticle by a co-precipitation technique carried at 310 ^{o}C, using toxic reagents like octadecene and oleic acid. Subsequently, epitaxial growth of $NaYF_4$ shell accomplished by dispersing the core nanoparticle in cyclohexane solvent, ultimately resulting in the fabrication of NaYF4:Yb^{3+},Tm^{3+}@$NaYF_4$ core-shell nanoparticle. Prepared core-shell nanoparticle is imparted hydrophilicity and surface functionalization by ligand exchange reaction. Three different lasers (namely: 1064 nm, 1150 nm, and 1208 nm) has been used as excitation strategies to obtain different response spectra of Tm^{3+} doped Upconverting nanophosphors. The 1150 nm laser excitation has enabled intense three-photon 475 nm emission, which is 100 fold stronger than 1064 nm excitation. A further arrangement of $NaYF_4$ shell layer significantly reduced luminesce quenching. The immunostaining

technique has enabled the HeLa cell microscopic imaging by using NIR-II excitation capability of thulium-doped nanoparticles.

Similarly, Chien et al. [190] have also synthesized Tm and Tm-Ho co-doped LaF_3 host lattice for the achievement of a bright Upconversion process. Hexagonal LaF_3 Upconverting nanophosphors resulted from the thermal decomposition of lanthanide oxides and trifluoroacetate precursors. During this synthesis oleic acid and 1-octadecene acted as coordinating ligand and non-coordinating solvent respectively. Interestingly by adjusting the NaOH concentration and reaction time period, Upconverting crystals with a single hexagonal phase and several morphologies such as hexagonal, rectangular, and irregular prism have obtained.

The Upconverting nanocrystals of calcium-deficient hydroxyapatite/tricalcium phosphate co-doped with ytterbium and thulium has been synthesized by Silva et al. [191]. The entire synthesis has been carried out using a co-precipitation technique in the aqueous phase under microwave irradiation (~900-1000 ^{o}C temperature) by adjusting the pH to 6. Prepared Upconverting nanophosphors resulted in efficient emission in visible (blue light) and NIR region (red colour). However, this technique has evolved particles having wide size distribution (up to 250 nm) and irregular morphology.

Generally as earlier stated, Upconverting phosphors have superiority over copper oxide or hydroxide-based nanomaterials on the basis of advanced photophysical properties. These advanced photophysical characters are highly recommended for probable use in multifunctional theranostic sectors. Additionally, unpaired electrons of doped lanthanide ion, enable thulium- doped Upconverting phosphors to be employed in various physical applications like optics and magnetism. Based on these attributes there are some earlier

reports on multifunctional thulium-doped Upconverting phosphors, which are reviewed in table 2.4.

Table 2.4 Literatures based on multifunctional Tm^{3+} doped Upconverting phosphors.

Sl no.	Thulium-doped Upconverting phosphors	Morphology & Size	Synthesis route	Multiple usability	Reference
1	Ba_2GdF_7:Yb^{3+}, Tm^{3+}	Sphere (~33 nm)	Hydrothermal	Up/downconversion emission of blue light and paramagnetic character	[192]
2	Hydroxyaptite:Gd^{3+}/ Yb^{3+}/ Tm^{3+}	Elongated hexagonal prism (~μm scale)	Hydrothermal	Paramagnetism and multimodal imaging of dental pulp stem cells via Up/downconversion luminescence	[193]
3	$Na_2Y_2B_2O_7$: Yb^{3+}, Tm^{3+}	Agglomeration of nanostructure with size ~20 nm	Solution combustion	Optical nanoheater, Optical temperature sensing	[194]
4	$Ba_5Gd_8Zn_4O_{21}$: Yb^{3+}, Tm^{3+}	NA	Sol-gel	Optical heating, Thermometry	[195]
5	NaYF4: Tm@NaYF, NaYF4: Yb/Tm@NaYF4	Core-shell structure (~20-25 nm)	Co-precipitation	Cancer cell imaging and spectroscopic properties	[189]
6	Y_2O_3: Tm, Yb	Nanophosphors	Homogenoous precipitation	Cathodoluminescence and Upconversion luminescence imaging of cancer cells	[196]
7	$NaGdF_4$:Yb^{3+}/Tm^{3+}	Spherical (~13 nm)	Thermal decomposition	Hemolysis evaluation, multimodal imaging of cancer cells	[197]

2.5 Summary of Entire Literature Review & Research Gap

The full focus in this literature review is centered on the multifunctional aspects of nanomaterials based on both copper oxide/hydroxide-based biominerals and rare earth doped Upconverting phosphors. The multifunctional prospects of the above nanomaterials are majorly aimed in cancer theranostics and diverse physicochemical fields. Therefore, Atacamite and copper oxide biominerals along with Tm^{3+} doped Upconverting phosphors have been investigated in this study within the nanoscale dimensions. There are not many reported research articles related to the multifunctionality of Atacamite nanomaterials especially in the field of advanced cancer theranostics as compared to copper oxide. Whereas there are a number of researches on copper oxide nanomaterials that demonstrate their simultaneous applicability in diverse sectors of biotechnology, catalysis, environmental pollution degradation, and energy. However the majority of the earlier adopted chemical routes for the synthesis of Atacamite (Table 2.1) and copper oxide nano/microstructures are not concerned about the hazardous effect from the use of toxic chemicals and subsequent stabilization process [198]. Additionally, maximum of the described techniques are labor-intensive, expensive, generate toxic byproducts [118, 199]. Most importantly, the adsorption of poisonous and non-polar reagents on the nanomaterial surface during synthesis limits their usage for application in the clinical field [200, 201].

Similarly, all kinds of literature reviewed for the successful synthesis and diverse functionality (Table 2.4) of Tm^{3+} doped Upconverting phosphors, are not concerned about environmental implications of the adopted strategy. Further, the reported methods rely upon the use of toxic, corrosive chemical reagents besides the high-cost factor. These are already described in the introduction section. Additionally, for precise control over the phase and

morphology of Upconverting crystals, template or organic additives like oleic acid, ethylenediaminetetraacetic acid, CTAB, ethylene glycol, etc. are employed [202, 203]. These expensive, and corrosive precursors limit the use of synthesized phosphors in the biomedical engineering field. Further, the conventional techniques adopted for both synthesis and surface functionalization by using chemical reagents need to be carried over a long time and at an elevated temperature.

Since there is a need for multiple usability in biological as well as diverse physicochemical applications, synthesized nanomaterials should be biocompatible and environmentally benign. The use of green synthesis techniques such as employing plant phytochemicals could be a feasible solution that may impart biocompatibility and multifunctionality to both semiconducting copper biominerals and Upconverting phosphors.

The plant extract, rich in polyphenolic and flavonoid substances, is widely used in nanotechnology as a stabilizing, nucleating agent. Synthesis involving plant extract seems to be advantageous over other biological species (bacteria/fungi) due to associated toxicity and difficulties involving in microorganism's isolation and incubation [204]. Further the greater affinity of the hydroxyl groups of polyphenols present in plant extract towards the base metal cation is an additional advantage [117, 118, 198, 205]. The phytochemicals in the plant extract, assist in regulating the morphology and physicochemical properties of developed nanomaterial for multifunctional applications. Furthermore, the synergistic effect of ascorbic acid, flavonoids, reducing-sugars present in plant extract imparts hydrophilic character to synthesized nanomaterial [206, 207], which is pivotal for biological usage. It is essential to mention here that the flavonoids content in plant extract has dual functionality. In association

with reducing-sugars, it performs as a hydrophilic entity, while in coordination with fatty acid esters, it turns out to be hydrophobic [208].

Improved circulation or half-life duration and augmented tumor uptake are related with hydrophilic and hydrophobic characters of nanomaterial respectively [5]. Flavonoids, vitamins, proteins, organic acids, carotenoids, and long-chain fatty acid ester enriched plant extract help in the nucleation and stabilization process during synthesis and minimize additional surface engineering steps for targeted theranostic bio-analytical applications [209].

Among all the biominerals and Tm^{3+} doped Upconverting phosphors currently under study, there is only literature available for green synthesis of copper oxide nanomaterials vastly synthesized using plant parts. Hence during the literature review for copper oxide (Table 2.2 & Table 2.3), with specific objective of multiple functionalities, synthesis based on plant parts have only considered.

As reflected in table 2.3, most of the green synthesized copper oxide-based nanomaterials mainly focused on their multiway applicability in various biotechnological fields. These sectors mainly include antimicrobial, biocidal, antioxidant potency of these semiconducting nanomaterials. In addition to this biotechnological efficacy, the potential of copper oxide nanomaterials in human biology, such as displaying cytotoxicity towards tumor/cancer cells, have also been investigated. The potential use of copper oxide nanomaterial as a probable chemotherapeutic agent specifically highlighted in table 2.2. Besides the cancer therapeutic use as a "chemotherapeutic agent," the multifunctional property of copper oxide nanomaterial has also propelled parallel research in the field of photocatalysis pertaining to

their improved photo-physical properties. As a whole, it can be understood that green synthesis may enable a technological leap by which a single unified platform can perform multiple chemical and biological objectives. In addition to copper oxide, copper hydroxide-based semiconducting nanomaterials have also been investigated by several other groups of researchers for multiple functionalities (Table 2.3). However, the researches related to the useful synthesis of copper hydroxide-based nanomaterials are very much limited. Moreover, the majority of reported researches involved use of toxic chemical reagents during synthesis and prepared nanomaterials are endowed with mono-functional characters (e.g. optical response, structural information).

On the other hand, in their research, Dong et al. 2020 [174] have highlighted the multiway usability of copper hydroxide nanoparticles as an antibacterial and biocidal agent. Still, again this work is based on a complex chemical synthesis route, with the production of bigger-sized particles. Further maximum of reported researches on copper hydroxide nanomaterials are devoted to antimicrobial applications rather than using them as possible cancer theranostics agent. In their research, Guo et al. [176] have reported the successful application of $Cu_2(OH)PO_4$ quantum dots in multimodal cancer theranostics applications. But the synthesis method adopted is based upon an expensive chemical route for the production of hydroxide-based semiconductor with additional constraints related to quantum dots toxicity and poor physical properties. As the green synthesis of nanomaterials using plant phytochemicals is one of the major objectives, therefore two of such researches on copper hydroxide nanomaterial led by Awwad [171] and Reddy et al. [172] have also been reviewed. Unfortunately, these two pieces of research have displayed only mono-functional attributes

of the developed nanomaterial. Unfortunately, these two pieces of research have displayed only mono-functional attributes of the developed nanomaterial.

Copper oxide or hydroxide-based nanomaterials till now discussed heve been synthesized from various plant parts (leaves, fruits, seeds, etc.) [210]. The phytochemicals (e.g., flavonoids, polyphenols, vitamins, ascorbic acids, etc.) responsible for the nucleation and stabilization [211, 212] of metal oxide/hydroxide-based nanomaterials are abundantly available in plant leaves. Further the extraction of phytochemicals from the plant leaves is more straightforward. Thus leaf extract is a promising candidate for the synthesis of copper oxide or hydroxide based biominerals [206]. Unfortunately, there is no work till now on the fabrication of nanoscaled Atacamite biomineral in the laboratory using plant leaf extract. But two of the researches carried out by Awwad et al. [171] and Reddy et al. [172] as earlier highlighted, described the synthesis of copper hydroxide based biomineral in lab scale and examined their antibacterial as well as physicochemical characterizations. However, there are plentiful research works regarding synthesis of copper oxide nanomaterials with diverse morphology and applications by employing plant leaf extract as an organic template [118]. These green synthesized copper oxide nanomaterials exhibit simultaneous functionality in various fields like nanomedicine, biotechnology, environmental remediation, and catalysis, etc., as explained in table 2.2 & 2.3. Moreover, their specific application as antimicrobial and photocatalysis agent are briefly described below.

Gowri et al. [211] have synthesized spherical copper oxide nanoparticles from the leaf extract of *Eupatorium odoratum*, *Acanthospermum hispidum* and studied their comparative antimicrobial behavior towards pathogenic bacteria. Aqueous phase reaction produced

irregularly shaped particles with its spherical equivalent considered for application purposes. In another study vanathi et al. [212] have prepared copper oxide nanoparticle from leaf extract of aquatic weed *Eichhornia crassipes* under vigorous heating over 8h time period. Prepared spherical nanoparticles (~30 nm) have been investigated for their antifungal activity against plant pathogens. Furthermore, Praburaman et al. [213] in their study have reported the inexpensive and eco-friendly synthesis of copper oxide nanoparticles using *Piper betel* leaf extract. Prepared nanoparticles are reported to be spherical in shape, with average diameter ranging up to 100 nm. They had inhibited the growth of plant pathogens *Ralstonia solanacearum* and *Xanthomonas axonopodis* besides exhibiting cytotoxic to *rat splenocytes*. Further copper oxide nanoparticles (~60 nm) are successfully developed using the leaf extract of *Populus ciliate* as a stabilizing agent [214]. The developed crystalline nanoparticles have demonstrated antibacterial activity for both gram-negative and positive bacteria species. Further copper oxide nanomaterials are essential class of semiconducting material and optically active, hence have helped to carry out photocatalytic degradation of waste matters when impinged with the light of suitable energy. Several researchers harnessed this potential of copper oxide nanomaterials economically synthesized from plant leaf extract in their research are described below. The Congo red and Safranin O dye degradation potential of copper oxide nanoparticles synthesized from leaf extract of *Kalopanax pictus* is investigated by Moon et al. [215]. The CuO nanospheres synthesized from the solution phase reaction carried over 24h time period have been found to be 50 nm in size. Furthermore, Rafique et al. [216] have carried out the green synthesis of copper oxide nanoparticles (~70 nm) from leaf extract of *Citrofortunella microcarpa* for photocatalysis purpose. Prepared nanoparticles are employed in the effective photocatalytic degradation of Rhodamine B dye

from wastewater with 98 % efficacy. In another study, Sankar et al. [217] have carried out the green synthesis of copper oxide nanoparticles from *Carica Papaya* leaf extract that is irregular in morphology and amorphous in nature. Prepared nanoparticles are effective in photocatalytic degradation of brilliant blue dye. Furthermore, Singh et al. [218] have synthesized CuO nanoparticles from leaf extract of *Psidium guajava* using copper acetate as precursor material. Resulting monodispersed small-sized nanoparticles (~6 nm) are reported to be successful for the effective photo-degradation of NB and RY160 dyes. There are no reports till now on Tm^{3+} doped Upconverting phosphors economically synthesized using plant leaf extract.

From the entire literature review on multifunctional "Atacmaite", "Copper oxide" and "Tm^{3+} doped Upconverting phosphors" which are preferably synthesized using plant leaf extract, one specific thing has been observed. The majority of researches are focused upon the biotechnological and environmental aspects of the synthesized nanomaterials. Further in cancer theranostics rather than extensively exploring in advanced process of bio-imaging and photothermal therapy, these nanomaterials are only investigated for their *in Vitro* toxic effect on cancer cells (Table 2.2). Nevertheless, apart from one or two reported researches [219, 220], there is no work regarding the use of plasmonic Copper oxide/hydroxide based nanomaterials and Tm^{3+} doped Upconverting phosphors as a photothermal agent for tumor ablation. Further as earlier stressed, in case of Tm^{3+} doped Upconverting phosphors for achieving best possible efficacy in cellular imaging and photothermal therapy the modulation in the morphology, crystallinity and luminescence properties are very much necessary. Hence research is now focused on using green synthesis of Copper oxide/hydroxide based biomineral and Tm^{3+} doped $NaYF_4$ Upconverting phosphors having multiple functionalities

in cellular imaging, photothermal therapy besides advanced physicochemical functions like electrochemistry or magnetism. Similarly, controlling the morphology, crystallinity and other photo-physical properties of inorganic nanomaterials is an indispensable part of multifunctional research.

2.6 Use of *M.oleifera* plant in the synthesis of Multifunctional Nanomaterial

The *M. oleifera* (family: Moringaceae) is an widely available plant in the Indian subcontinent and has wide therapeutic usage in microbial, cancer, inflammation, diabetics-related diseases [221, 222]. Furthermore, *M.oleifera* leaves are known to enrich with flavonoids, polyphenolics, phenolic acids, vitamins, proteins, carotenoids, and glucosinolate, polypyrrole, and long-chain fatty acid ester compounds [223-225]. Detailed analysis of phytochemicals present in *M.oloeifera* are described in table 2.5. These bio-molecules/phytochemicals are known to facilitate "size control" and "promote stability" [226-228]. "Flavonoids", is one of the important phytochemicals act as effective reducing and stabilizing agent during synthesis of nanomaterial and found in highest quantity in *M.oleifera* leaves, in comparison to other available medicinal plants found in india [229]. In addition to, since *M.oleifera* leaf extract actively participate in the nucleation and growth process of transition metal-based nanomaterials [226, 228, 230] discussed in the current study, they could be effectively employed in synthesizing rare earth doped Upconverting phosphors also. The long-chain fatty acid esters abundantly present in *M.oleifera* extract could be ideal for $NaYF_4$ Upconverting phosphor synthesis instead of previously mentioned chemical chelating agents. Overall, *M. oleifera* leaf extract could potentially facilitate the environmentally benign and cost-effective synthesis of divalent copper-based biominerals

and trivalent thulium-doped Upconverting phosphors, restraining the use of corrosive expensive nucleating/stabilizing agents. In fact, *M.oleifera* leaf extract has the utmost potential to drive the research related to size and morphology tuning of semiconducting copper-based biominerals and Tm^{3+} doped $NaYF_4$ Upconverting phosphors. Moreover, rich phytochemicals present in *M.oleifera* can impart surface functionality to above-prepared nanomaterials in a facile manner for diverse biomedical, physicochemical usage by not using the complex ligand exchange reaction.

Based on the above mentioned attributes, there are reports on ultra-small gold (3-5 nm) [231] and ~10-30 nm-sized semiconducting nanoparticles [225, 232, 233] by using *M.oleifera* plant extract. The small-sized (~12 nm) Copper oxide nanospheres are also synthesized from *M.oleifera* extract. These nanosphers are reported to have toxic effect on several cancer cell lines such as breast (MCF-7), cervical (HeLa), epithelioma (Hep-2), and lung (A549), etc [160]. Hence the *M.oleifera* assisted nanomaterial can be effectively promoted in cancer theranostics.

Table 2.5 Common phytochemicals present in *M.oleifera* leaf extract [234, 235]. $^{\#}$Gallic acid equivalent, $^{@}$Quercetin equivalent, $^{\$}$Tannin acid equivalent.

Bioactive group	Compounds	Concentration (mg/100 g)
Vitamins	Vitamin A	0.37
	Thiamine	0.06
	Riboflavin	0.05
	Niacin	0.8
	Ascorbic acid	220
	Vitamin E	9

Carotenoids	β-carotene	6.8
Polyphenols	Total phenol	10,504[#]
Phenolic acids	Caffeic acid	40.9
	Chlorogenic acid	48.9
	Ellagic acid	18.9
	Ferulic acid	12.8
	Gallic acid	103.4
Flavonoids	Total flavonoid	3128[@]
	Isorhamnetin	0.4
	Quercetin	16.6
	Kaempferol	6
Tannins	Total tannins	13.2[$]

Most importantly, the abundant availability of *M. oleifera* throughout the year on the Banaras Hindu University campus is indeed an added advantage.

2.7 Biomineralization: A Possible Mechanism for MFN synthesis

The copper-based semiconducting nanomaterials engrossed in this research were earlier designated as biominerals since their synthesis process mimics natural bio-mimetic mineralization technique. Biomimetic mineralization simulates the natural mineralization process in the aqueous environment and produces biominerals with controlled size, morphology, and characteristics by a synergistic combination of organic and inorganic substances [236]. It is indeed best suited method to go hand-in-hand with *M.oleifera* leaf extract for synthesis of multifunctional nanomaterials under green synthesis approach [237,

238]. Further by following this technique high-quality thulium-doped UCPs can be synthesized, which have controlled crystallinity, phase, morphology, and photo-physical properties. For lab-scale biomimetic mineralization, techniques such as sol-gel, microemulsion, and hydrothermal are generally adopted. But most importantly, the hydrothermal method provides a single-step versatile approach for synthesis of phase pure mineralogical structure from their heterogeneous precursors [239]. Similarly, the facile hydrothermal route has put forward a moderate (temperature employed: 140–240 ^{o}C) reaction condition, which allows the use of various low boiling ligands having low cost during synthesis of Upconverting phosphors [240]. Interestingly with slight modification in chelating ligands and growth controlling substrates, Tm^{3+} doped $NaYF_4$ Upconverting phosphors with assorted morphology, dimension, and optical characteristics could be achieved via a one-step hydrothermal method.

In the hydrothermal synthesis, crystal nucleation and subsequent growth proceeds through solubilization of precursor material and corresponding chemical reactions under elevated pressure and temperature relative to ambient conditions inside a sealed container. This process indeed mimics the natural mineralization technique. The careful control of external (energy provided) and internal input variables (temperature, pH, time, the volume of reactant, organic precursor, templates, etc.) is the key to the successful synthesis of various nanomaterials. Based on the adjustment of internal parameters, hydrothermal synthesis can be categorized into a template, or organic additive assisted synthesis, the combination of both, and synthesis free of template or additives, and lastly substrate assisted. By Using *M.oleifera* leaf extract as an organic additive, the synthesis of both nano sized crystalline

minerals and Upconverting phosphors under hydrothermal method could be the best choice for its reduced cost, easy surface functionalization, and simplistic approach.

Moreover, the hydrothermal route described above allows the production of size & morphology tunable nanomaterials with ample surface defects [241, 242]. These surface defects act as a probable heat-generation sites upon light exposure [243]. The photo-generated heat could facilitate an effective photothermal therapy for the treatment of tumors.

All these discussions lead to the conclusion that the synergistic combination of hydrothermal method [119] and *M.oleifera* leaf extract facilitates the generation of nanomaterials based on copper-based biomineral and Tm^{3+} doped $NaYF_4$ Upconverting phosphors. Further there could production of nanomaterials having controlled size, shape, and crystalinity. Additionally, by following this lab scale biomineralization technique, a single step, and low-cost mechanism might be developed, which could lead to advanced physicochemical characteristics and achievement of improved surface functionalization for cancer theranostics. Overall it can be stated that the biomineralization process involving use of plant leaf extract for the synthesis of Atacamite, Copper oxide, and Tm^{3+} doped Upconverting nanomaterials are a noble and inexpensive technique not endeavored earlier.

2.8 Major Objectives of the Current Research

- The main objective of the current study is to develop the multifunctional nanomaterial out of biominerals namely Atacamite and Copper oxide along with Tm^{3+} doped $NaYF_4$ Upconverting phosphors for investigating their potential in cancer theranostics by integrating multimodal imaging, photothermal heat generation attributes. Further, these nanomaterials are proposed to be investigated in diverse physicochemical applications also.

- The multimodal imaging mainly comprises of luminescence and MR imaging contrasts. Similarly, photothermal heat generation capability of these nanomaterials upon exposed with NIR (975 nm) light, are proposed to be investigated in the cancer cells grown in Vitro.

- Besides theranostics, the multiway usability of these nanomaterials is proposed to be explored in terms of investigating their magnetic, photo-physical and electrochemical characteristics. Such that additional objective for development of MRI contrasting agent, optical devices and electrochemical energy storage or bio-sensing can be fulfilled.

- Most importantly in view of possible biological applications, these inorganic nanomaterials are proposed to be synthesized from a green route (specifically using the plant extract) by following the biomineralization technique. Additionally, for effective biomedical usage, the morphology, crystallinity, and luminescence of Tm3+ doped UCP are also proposed to be controlled by variation in the basic synthesis components.