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MINT PLANT MEDIATED NANO-PARTICLES SYNTHESIS, CHARACTERIZATIONS, AND APPLICATIONS: A REVIEW

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Abstract: The nanotechnology has impacted all human scientific and technological developments in recent time. Innovative applications of the nanoparticles have been implemented in practice. The synthesis of nanoparticles involved multiple chemicals and byproducts. The chemicals involved and products generated might be hazardous for environment. The mint plants have their importance since Vedic era. In this review authors have tried to review the green synthesis of nanoparticles using mint plants. Authors review adopted synthesis methods, general characterizations performed and the applications of the synthesized particles. Review involves nearly decade long research work on mint plant mediated nanoparticle synthesis. Extensive discussion and future scope of the reviewed articles has been put forth which will be helpful for the researchers in future.

Keywords: Mint plant, Nanoparticles, Antibacterial, Antifungal, Antioxidant

1. INTRODUCTION

The nanotechnology has reshaped many dimensions of human life. The enormous applications of nanoparticles (NPs) make these inevitable in today's life. NPs are used in sensors, fertilizers, biomedicines, coatings, water purification, dye degradation, energy storage, antibacterial and antimicrobial applications [1].

As the science and technology is advancing daily at rapid rate, the side effects of the processes are also needs to be addressed. The chemicals used in synthesis processes also generate pollutants. The use of bio-reduction for synthesis of NPs has been an area of interest for many research groups. These methods use extracts from various parts of the plants (i.e. roots, stem, leaves, flowers, seeds, and seed covers etc.) as the reducing agent [2]. The use of bio-reduction might decrease the hazardous products of the processes.

Fig. 1 The menthe family species

The mint family plants have been historically used in household cooking in India. The spearmint (Mentha Spicata), peppermint (Mentha Piperita), water mint (Mentha Aquatica), wild mint (Mentha Arvensis), Pennyroyal (Mentha Pulegium), Mentha Suaveolens, Mentha Canadensis l., and Mentha Longfolia, etc. are the sub-species under the genus mentha. [3] Mint plant has been also used for the medicinal purposes. The aromatic leaves of mint plant are also a daily ingredient of Indian kitchens. The leaves and stems of mint plant have resinous dots containing volatile oils. The phytochemical analysis of ethanolic leaf extract of mentha arvensis revealed and confirmed the presence of alkaloids, terpnoids, phenolics, proteins, and carbohydrates. [4]

The review of the decade long investigation on the Mentha based nanoparticle synthesis might be useful for future research endeavors. Authors chose to review the use of mentha family plants for synthesis of NPs. The available research articles since 2014 were collected, analyzed and reviewed rigorously. The keywords like biosynthesis from mint plant, nanoparticle synthesis from mint plant, nanoparticle synthesis from mint plant extract were searched on Google scholar, Research gate etc. Around 50-55 research abstracts / articles were available. Authors tried to review the synthesis methods, the characterizations used and the applications of it. Nearly 50 percent of the articles have used multiple characterization techniques, to generate solid base for the proper research conclusions. Generally the characterization techniques like x-ray diffraction (XRD), field effect scanning electron microscopy (FE-SEM), Fourier transform infrared spectroscopy (FTIR), ultra-violet visible absorbance spectroscopy (UV-Vis), and energy dispersive x-ray analysis (EDAX) have been adopted by the authors.

Majority of the research articles reported synthesis of silver (Ag), gold (Au) NPs using mint plant extract. Few articles have reported bio reduction to zinc oxide (ZnO), Titanium oxide (TiO_2), Iron (Fe), Calcium oxide (CaO), chitosan, Copper (Cu), and Selenium (Se) NPs [5, 6]. The diverse sizes and shapes of the NPs were analyzed and reported. Commonly the NPs were used in antibacterial, antifungal and antimicrobial applications [7]. To track exact working principle behind the various antimicrobial performances still remains the challenge in front of the researchers. In depth analysis of the antifungal, antibacterial, and antifungal activities must be attempted in future.

2. NPS SYNTHESIS METHODS

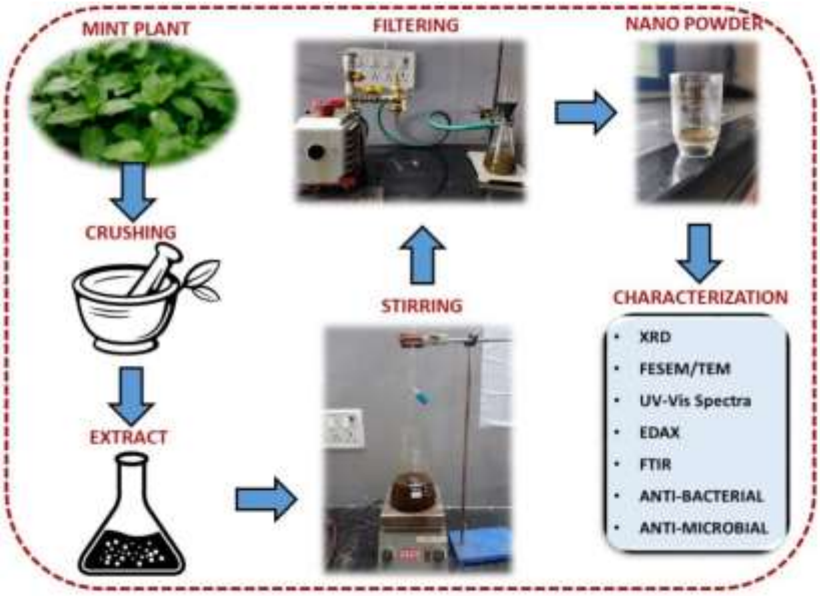


Fig. 2 General work flow for the green synthesis of NPs

The general bio-reduction / biosynthesis / green synthesis method employed for synthesis of multiple NPs by the majority of the researchers can be summarized in fig. 2. The aqueous extract of parts of the mint plants can be prepared using desirable ratios of mint leaf and double distilled water (DDW). The leaves are crushed and mixed with DDW. The solution is heated using hot plate for average 10-20 minutes. The extract is filtered and used for reduction of preferable the salts of desirable NPs. The reaction parameter i.e. stirring temperature, stirring speed, stirring time, pH of the solution, and concentration of the salt solution determine properties of the colloidal NPs. Further the colloidal NPs can be filtered using vacuum filtration setup. The NPs obtained can be purified by further washing and used for characterization.

The color changes during the colloidal solution formation itself give the hint regarding the NPs formation. The plant extract helps in reduction of metal salt [8]. Various characterization techniques are employed according to the desired application of the NPs. This green synthesis technique generates lesser chemical pollutants as compared to the chemical reduction technique [9].

3. DISCUSSION

3.1 Review of NPs synthesis using Mint species extract as a reducing agent:

The review of all the articles might lay down concrete path in front of the interested researchers for the future. Authors have reviewed the various research articles in chronological order of year of publication. UV-Vis spectroscopy is used by authors generally to assess the progress of the biosynthesis of various NPs. The involvement of various functional groups is studied using Fourier transform infrared spectroscopy (FTIR) technique hence we can observe regular use of it. The scanning electron microscopy (SEM) was utilized by the authors to understand the surface morphology of the synthesized NPs. The agglomeration properties can also be observed via SEM. The crystalline nature, crystal parameters, strain analysis was done by authors using X-ray diffraction (XRD) studies. The particles size and size distribution was confirmed using Dynamic light scattering (DLS) method.

Kumar SP et al. (2014) have synthesized the nano scale iron nanoparticles (Fe NPs) (20-45 nm) from mint leaves and studied their role in removal of arsenic (As III and As V) from aqueous solution. The Fe NPs were observed to have core-shell configuration. FTIR study revealed that particle materialization involved functional groups like -NH, -C=O, -C=N and -C=C. The Fe NPs and chitosan composite removed 98.79 and 99.65 % of As (III) and As (V) respectively. The adsorption studies (pseudo first and second order kinetic model) analyzed the role of contact time and adsorbent doses in removal of arsenic [10].

Gold NPs (52 nm) were bio-synthesized by Thanighaiarassu RR et al. (2014) using leaf essential oil of Mentha pieperita. Antifungal activities of the synthesized NPs were found to be efficient against human pathogenic fungi such as Candida tropicalis, Candida kefyr, and Candida albicans. Authors proposed that the presence of menthol in mint plant worked as a bio-reductant and in turn caused the antifungal behavior [11].

Antioxidant and antimicrobial behavior of mint derived nano silver NPs (75 nm) was studied by Marcela EBP et al. (2014). Authors used X-ray fluorescence (XRF) technique to confirm presence of silver NPs and chemiluminescence (CL) assay to determine antioxidant activity. Phyto-synthesis of silver NPs combined the benefits of silver NPs and *Mentha piperita* plant. The phyto silver NPs along with liposomes and carbon nanotubes (CNT) were found to be efficient against *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis.* According the authors addition of CNTs improved the antimicrobial activity of Ag NPs. More surface area of CNTs helps to perturb the cell integrity and hence damages the cell wall of bacteria causing its death. The concept of decorating the CNTs with multiple combinations of NPs and liposomes appears to be innovative and efficient technique to enhance antimicrobial activity. [12]

German AV et al. (2014) synthesized gold nanoparticles (Au NPs) (3-26 nm) using *Mentha piperita* extract. The effect of extract concentration, heating, pH of colloids and storage time on Au NPs was analyzed by the authors. Particle size and size distribution was studied using high resolution tunneling electron microscope (HR-TEM) technique and ImageJ software. The colloidal stability was analyzed by observing periodical Zeta potential (ZP) and UV-Vis spectrum of refrigerated sample over time span of 4 weeks. While the Au NPs were synthesized and studied thoroughly, no application oriented study was conducted by the group. [13]

Synthesis of Fe NPs using Holy basil and mint leaves extract was reported by Wanida W et al. (2015). UV-Vis spectra and cyclic voltammongram was used to analyze the Fe NPs. The application and other morphological parameters were not discussed in the article. [14] Shraddha AB et al. (2016) prepared Ag NPs using the mint extract with polyvinyl alcohol (PVA) as capping agent and used it in Chitosan/Gelatin composite packaging film. This was different kind of application reported. Surface Plasmon resonance (SPR) was used to confirm Ag NPs formation and its stability during storage. The homogeneity, mechanical properties and water vapor transmission rate of films increased due to the Ag NPs incorporation. The packaging films had antibacterial behavior against the *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus*. [15]

Ag NPs (50 nm) were prepared from mint leaf extract and studied for antibacterial performance by Avila MG et al (2017). The force atomic microscopy (AFM), transmission electron microscopy (TEM), Energy dispersive X-ray (EDX) and DLS were used by the authors. The total quantity of phenolics was also determined by the authors. The antibacterial activities were studied for *Escherichia coli* and *Staphylococcus aureus*. Minimum inhibitory concentration against E. coli was reported as 2.49 $\mu g.ml^{-1}$. The authors discussed the size of NPs as one of the cause for antibacterial activity. Toxic Ag^+ ions are attracted to negatively charged bacterial cell wall which modifies permeability of the cell wall. The ions interact with cytoplasm and ribosome to reduce ATP production and hence cause bacterial death. [15]

Mint (*Mentha piperita*) and Cabbage (*Brassica oleracea* var. *capitata*) leaves extract were used by Malwina S. et al. (2017) to synthesize Ag NPs. The Ag NPs had 5-50 nm size for mint and 10-150 nm for cabbage mediated process. The pH and temperature variation affects the size of the particles. The activity against microbes like *E. coli*, *S. aureus and S. enterica* was studies in the article. The antibacterial activities were assessed by metabolic assay- PrestoBlue and XTT test. Authors reported trans-plasma membrane electron transport as the reason behind the antimicrobial activity. [16]

The work on synthesis of Ag NPs from *Mentha arvensis* (*Linn.*) and its anticancer activity against PARP1, P53, P21, Bc12, Bax and cleaved caspase 9 proteins in MCF7 and MDA-MB-231 cells was reported by Prajna PB et al. (2017). The article reports cell-cycle analysis using fluorescence-activated cell sorting. Expression pattern of cell was analyzed using Western blot method. Ag NPs enhance apoptosis in MCF7 and MDA-MB-231 cells, hence Ag NPs exhibited cyto-toxic nature against breast cancer cells. [17]

Savita P et al. (2017) reported catalytic behavior of Ag NPs synthesized using Mint leaves. Authors used spectrophotometry under pseudo first order condition. The presence of Ag NPs improved oxidation of metronidazole (MTZ) by hexacyanoferrate (HCF). [18] A review on green synthesis of gold NPs was reported by Muhammad N et al (2017). It has not a single discussion of gold NPs using mentha species. [19] *Mentha piperita* mediated CaO NPs synthesis was reported by Ijaz AB et al. (2017). Agar diffusion method was used for antimicrobial assay. The minimum inhibitory concentration of CaO NPs was 25 $\mu g.ml^{-1}$ against *E. coli*.

[20] Diptendu S et al. (2017) reported use of *Metha asiatica* as a reducing agent to prepare Ag NPs and analyzed the antimicrobial activity of the same. Interaction of NPs with DNA could hamper replication and hence cause cell death. [21] *Mentha longifolia* extract was used to synthesize chitosan NPs (157 nm) by Abeer RMAE et al (2018). The antifungal activity of chitosan NPs was tested as effective against mycelium growth of *Aspergillus niger*. [22] Mint mediated biosynthesis of Ag NPs (26 nm) was also reported by Wisam JA et al. (2018). The NPs showed better antibacterial response against *Bacillus subtilis* and *Escherichia coli*. [23] Saurabh S et al (2018) reported synthesis of zinc oxide (ZnO) NPs using mint leaves extract as a reducing agent. Two precursors Zinc acetate and Zinc nitrate were used to prepare the ZnO NPs of size 29 and 32 nm respectively. [24]

A review on Ag NPs derived from medicinal plants (including *Mentha piperita*) was reported by Shahid UK et al (2018). This study focused on the action of Ag NPs against Honey bee pathogen. It reports that spearmint oil (derived from *Mentha spicata*) was effective against *Paenbacillus larvae.* [25] Wisam JA et al. (2019) prepared cupric oxide (CuO) NPs (22-25 nm) using copper nitrate precursor and mint leaf extract as a reducing agent. The antimicrobial activity was analyzed against *E. coli* and *Bacillus subtilis* [26]

Mentha arvensis extract was used by Bilal J et al (2020) to prepare Ag NPs. The effect of various reaction parameters like ratio of reactants, pH, temperature, concentration, and incubation period in reaction dynamics of formation of Ag NPs was discussed and neatly demonstrated in Fig.3. The effect of NPs on Colon cancer cell HCT 116 and cell cycle analysis was reported by the authors. Half maximum inhibitory concentration (IC_{50}) was analyzed for colon cancer cell. Authors proposed presence of chlorine in Ag NPs as the reason behind anticancer behavior. Chloride ions facilitate transmembrane flow of bicarbonates (HCO_3^-) i.e. chloride shift is responsible for the anticancer cell activity [27].

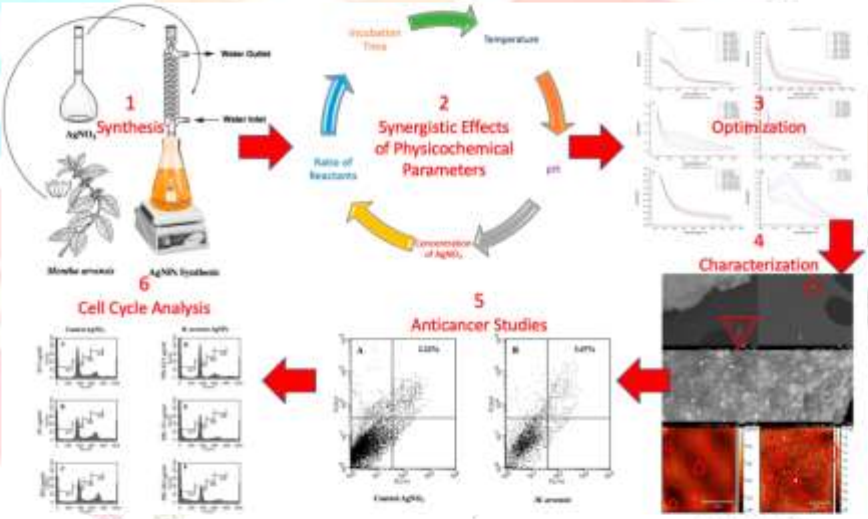


Fig. 3 1. Schematic of work flow, 2. Important physicochemical parameters, 3. Results of spectrophotometric analysis, 4. Characterization of nano-structures and 5. & 6. Biological applications of Biosynthesized Ag NPs [27]

(Note: Figure is adopted from ref no. 23. Article has open access and is under creative commons attribution non-commercial (unported, v3.0) license, it does not require special permission from the author.)

Bilal J (2020) used Mentha longifolia extract with silver nitrate precursor to prepare colloidal Ag NPs (20-100 nm) and reported its antimicrobial activity against plant bacterial pathogen. Antibacterial activities of Ag NPs against pathogenic bacterial strains were effective. The human red blood cells (RBC) were exposed to prepared Ag NPs and hemolysis assay was done. The response to plant bacterial pathogen was attributed to the reactive oxygen species, which perturb the transmembrane ionic balance and cause cell death. Ag NPs also bind with the thiol group (-SH) of mitochondria breaking homeostasis of cell and hence cell death. [28] Mansureh et al (2020) also synthesized Ag NPs (10-20 nm) from extract of *Eucalyptus obliqua* and *Mentha spicata*.Ag NPs prepared from mint extract were reported to be more stable [29].

Ahmed A (2020) reported green synthesis of zinc oxide (ZnO) NPs (11-88 nm) using *Mentha Spicata* extract. The antiviral activity of ZnO NPs against Tobacco mosaic virus (TMV) was studied by the authors. ZnO NPs treated tomato plants showed reduced TMV accumulation in plant tissues. Flavonoids, metabolites play major role against TMV. [30] Fatema MAA (2020) reported biosynthesis of Ag NPs and its antifungal activity against *Aspergillus niger* [31].

Disc diffusion techniques was used to test the antibacterial activity of TiO_2 NPs. TiO_2 NPs were found efficient against *Proteus vulgaris* bacteria owing to the dissolving of outer bacterial shell. In addition to that NPs exhibited antifungal activity versus *Aspergillus niger*. [32] Bilal J et al (2020) compared the Ag NPs (10-20 nm) prepared using *Mentha longifolia* and *Mentha arvensis* extract as a reducing agent. The NPs were reported to be within acceptable drug delivery size limit.[33] Biocompatibility of mints oil - *Vitamin D* encapsulated β-Cyclodextrin carbon-based NPs was analyzed by Faisal G et al (2021). The *L929* and *HeLa* cell lines were used to test cytotoxicity and anticancer activity of the NPs. The seed germination capability was unaffected, whereas root and shoot length of seedlings was affected by the use of NPs. The percentage survival, heartbeat rate and hatching assay of Zebrafish model system was also conducted by the authors. NPs were reported to be nontoxic at lower concentrations [34]. Iqra B et al (2021) used *Mentha asiatica* leaves extract to prepare silver NPs (27-31 nm). The antioxidant and cytotoxic potential of Ag NPs was also explored in the study [35].

ZnO NPs were synthesized via green route by Manjula R et al (2021). The Hall effect studies reported transition of NPs from n-type to p-type semiconductor due to increase in *Mentha arvensis* extract concentration. Photoluminescence study of the NPs propose them to be efficient for optoelectronic applications [36].

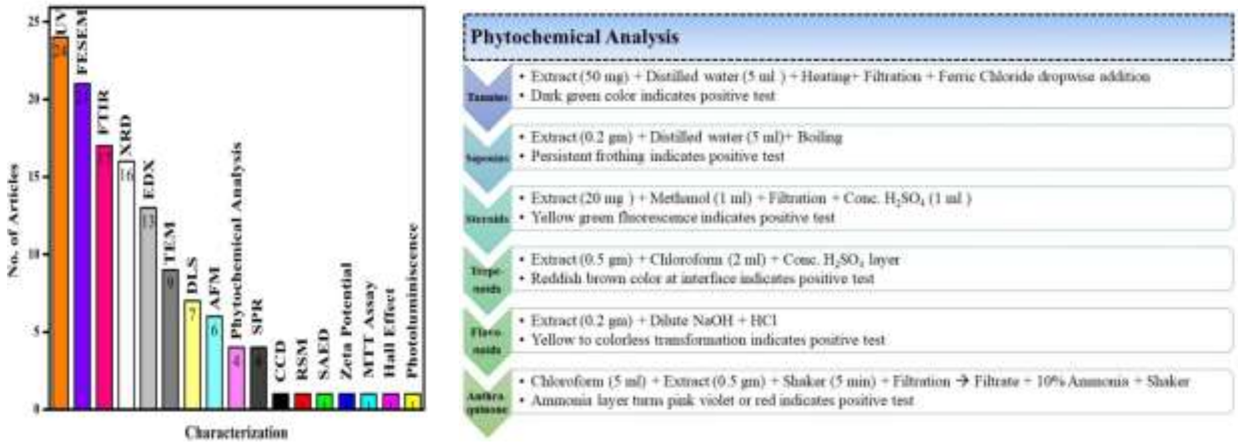
Copper (Cu) NPs were synthesized using copper (II) sulfate pentahydrate precursor using chemical reduction process by Sarab WA et al (2021). As compared to other research article, in this study Cu NPs were mixed with mint plant leaf extract, incubated and analyzed later. The *Cyprinus carpio L.* fish infected with *Saprolegnia spp.* were treated with Mint-Cu NPs. In depth analysis of effect of NPs on white blood cells (WBC), red blood cells (RBC), haemoglobin (Hb), total protein, albumin and globulin were reported. Mint-Cu NPs can work as an antifungal agent against *Saporlegniasis* [37]. Fatemeh K et al (2022) reported that the foliar spray of Seleium NPs improves yield, content of essential oils, and composition of *Mentha suaveolens* Ehrh. i.e. pineapple mint under salinity stress condition. [38] Kartikey K et al (2022) compared structural and optical properties of ZnO NPs biosynthesized from Mint (26 nm) and Neem extract (25 nm) [39]. Mariam M et al (2022) prepared films of *Mentha piperita* extract and Ag NPs. The in depth histopathological study of rat wounds with and without application of the gel of combination was reported. The synergistic properties of Mint and Ag NPs were investigated to be effective in wound healing in rats suffering from type I diabetes [40].

Mariam M et al (2022) synthesized Ag NPs using *Mentha piperita* L. leaves. The combination of polyvinyl alcohol, castor oil and corn starch with Ag NPs were used to prepare hydrogel films. The flexible and biodegradable gels were studied to be thermally stable upto 200°C, effective against *S. aureus* and *P. aerugnosa* and hence can be used as efficient wound dressings [41]. Santhanamari T et al (2022) employed *Mentha arvensis* Linn. extract as a bioreducing agent to prepare Ag NPs. The antibacterial behavior of Ag NPs was analyzed in terms of minimum inhibitory concentration of 250 $\mu g.ml^{-1}$ against gram positive bacteria [42]. Mahdjoube GM et al (2022) used biosynthesized Ag NPs from *Mentha piperita* leaf extract in glass ionomer cement. The composite was tested for antibacterial activity against five different bacteria by observing the growth inhibition zones. Highest antibacterial effect was reported against L. acidophilus [43].

3.2 Characterizations:

Fig. 4a demonstrates the characterizations used by the researchers. The most common characterizations are the UV-Vis, SEM, FTIR and XRD. The structural parameters (crystallite size, most preferred orientation, lattice parameters etc.) are generally analyzed using XRD. In some special case the XRD analysis can be used to analyze the stress, strains developed during the synthesis process due to annealing, grinding etc. [44]. The surface morphology, particle shape, amount of agglomeration, and particles size distribution affect the performance of the synthesized NPs, hence the SEM has been used quite often [45].

The most used characterization is the UV-Vis. The process of nanoparticle formation can be monitored and confirmed using UV-Vis. Molecular size of the NPs is also determined by UV-Vis. The changes in molecular structure during the bio-reduction (using plant extract) can be examined by taking UV-Vis spectrum at the regular intervals [43]. The presence of various compounds can be identified and confirmed using FTIR. The bonding type, strength, and probable reaction kinematics can be assessed by FTIR spectrum [46]. Along with these characterization the antibacterial, antimicrobial and antifungal tests have also been carried out by most of the authors.



(a) (b)

Fig. 4 a) The number of articles using various characterizations **b)** Methods implemented for phytochemical analysis during green synthesis of silver NPs

The most used characterization lay down the foundation for the more specific (application oriented) analysis. The lesser used characterizations are intended for particular application. These applications are more application oriented / target specific. As the production of NPs is carefully analyzed so should be the effects on bacterial cell. Especially the cell cycle analysis adopted by 8 & 23 appears to be more important in addition to the basic characterizations if authors are targeting the antibacterial / antimicrobial activities.

Waseem A et al (2020) used *Mentha arvensis* extract and titanium tetraisopropoxide precursor to synthesize titanium dioxide (TiO_2) NPs (20-70 nm) [32]. Authors carried out phytochemical test which are summarized in Fig. 4b.

3.3 Applications:

Table I demonstrates the information of the Mentha species used for extract formation, the NPs synthesized in the bio-reduction and percentage of articles using particular characterization. As the different mentha species are found in different regions of the world, the proportion of phytochemicals in mentha extract is/might be different [47]. Most authors have used the extract of the Mentha piperita and Mentha arvensis. Actually the comparative phytochemical proportions in different Mentha species still appear to be unexplored. This comparative analysis could lay down tangible basis for the application oriented use of the Mentha species.

54% of the articles have reported the use of mentha extract for biosynthesis of Ag NPs. This is obvious due to the effectiveness of the Ag NPs against the microbes, bacteria and fungi. The Ag NPs have been historically used for the applications like food storage packaging, cosmetics, coatings of textiles, detergents, water treatment, and wound healing gels etc. Few of the authors have synthesized ZnO, Au, CaO, TiO_2 and Chitosan NPs. Instead of using solo NPs, the combination with glass ionomer [43], chitosan [22] and carbon nano tubes (CNTs) [12] could widen the application spectrum.

Table 1. Percentage of articles using various Mentha species, NPs and characterizations

Sr. No.	Mentha Species	Percentage of Articles	Nano Particles	Percentage of Articles	Characterization	Percentage of Articles
1	*Mentha piperita*	26	Ag	54	UV-Vis	69
2	*Mentha arvensis*	20	ZnO	11	SEM	60
3	*Mentha spicata*	9	Chitosan	9	FTIR	49
4	*Mentha longifolia*	9	Au	9	XRD	46
5	*Mentha asiatica*	6	CaO	3	EDAX	37
6	*Mentha aquatica*	3	TiO_2	3	TEM	26

Mechanism behind the applications:

The mechanism for antimicrobial activity of the nanoparticles has been summarized in figure 5.

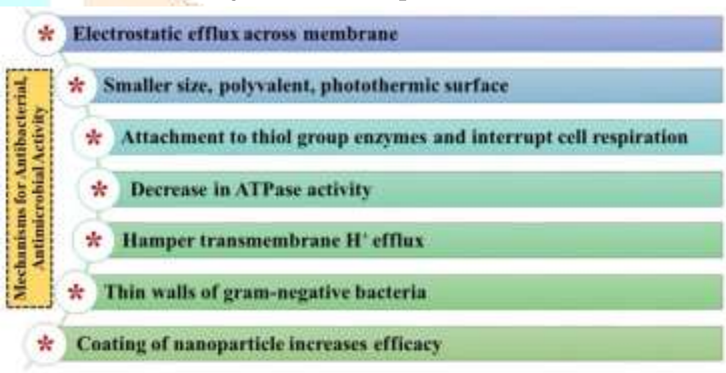


Fig. 5 Mechanisms discussed for antibacterial, antimicrobial activities of NPs [12, 13, 15, 28]

The cell membrane penetration depends upon the shape and size of the NPs. In decreasing order, the shape based penetration probability is for triangular, spherical and lastly nanorodss. The more number of active facets are available in case of triangular NPs and hence has more reactivity. The NPs are effective against gram negative bacteria as it has relatively thin wall as compared the gram positive bacteria.

The mechanism behind the performance of NPs as an antibacterial, antifungal, and antimicrobial agent have been discussed by some of the authors. The extract of the discussions has been summarized in fig. 5. The NPs (< 10 nm) can directly interact with microbe, interfere the microbe cell metabolism and destroy it. The NPs bind with thiol group (-SH) in the respiratory enzymes, generate reactive oxygen species and suppress its respiratory activity causing cell death. [48] The NPs get attached to phosphorus containing compound (Di-nucleic acid i.e. DNA) and hamper the reproduction of the bacterial cell [32]. The interaction of NPs with RNA i.e. ribonucleic acid inhibits the protein synthesis and hence causes cell death. If the NPs get oxidized, the transmembrane potentials are altered. The changes in trans membrane electrostatic efflux facilitate the entry of the NPs inside bacterial cell.

4. CONCLUSION AND FUTURE SCOPE

- In piercing the cell wall rather than spherical NPs and nano rods, triangular NPs play a major role. In addition to the transmembrane electronic efflux the intersecting sharp corners of triangular NPs could facilitate easier entry inside the cell.[19]
- The use of protective agent / surfactant avoids the NPs agglomeration. The stable dispersive NPs are very important for its effectiveness against the bacteria/microbes/cancer cell. The stable dispersive NPs could lower drug dose concentration and hence can decrease the side effects. The use of biogenic protective/capping agents (e.g. starch, thiols, amines, acids and alcohols) is still the hurdle. [49, 18, 37,41]
- Some articles have reported NPs biosynthesized using mentha extract for use in antifungal chitosan coating film application. In such reports, steric effects (steric hindrance) need to be analyzed in depth. Use of biogenic capping agent along with NPs could also be useful in this case. [50]
- Antimicrobial tests are almost concluded after finding out the inhibition zones but the study can be further extended to find out the type of antimicrobial behavior (bacteriostatic, bacteriocidal, and bacteriolytic) of the synthesized NP.
- Most authors stated that the toxic chemical waste is reduced. The comparative estimate of the reduction in toxic chemicals could stamp the importance of use of mentha extract
- The shift from laboratory research to mass production remains the major task.

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