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Review Article

Bioinspired Synthesis of Nanoparticles and their Biomedical Potential: the Pakistan Experience

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Abstract: Nanotechnology is an emerging field that play pivotal role in a wide range of scientific fields. Applications of nanotechnology have been successfully applied in healthcare, drug delivery, gene delivery, diagnostics, and energy sciences. Nanoparticle synthesis involves different methods like physical, chemical approaches and biological methods. The physical and chemical methods are associated with pitfalls as they pose potential threat to the environment; and hence ecofriendly routes of bioinspired nanoparticle synthesis are preferred. The biogenic synthesis of nanoparticles has attracted numerous researchers because of their potential advantages such as simplicity, safety, easy production, biocompatibility, and low production costs. Green synthesis of nanoparticles involves the mixing and processing of metal salts with plant/bacterial/fungal extracts. Secondary metabolites from biological sources have potentials for reducing the metal salt(s); herby synthesize respective nanoparticles. The current review is aimed to discuss reports and studies conducted in Pakistan that have used biological approach for nanoparticle synthesis, as well as their potential biological and pharmacological application/s. Future directions should involve market oriented approaches for the commercialization of nanoparticles-based products that can help in up-left of national economy.

Keywords: Nanoparticles; Green synthesis; secondary metabolites, Pakistan

1 INTRODUCTION

Nanotechnology is an important field of modern research that deals with the synthesis, designing and manipulating particle structures having size from 1-100 nm [1, 2]. The synthesized nanoparticles (NPs) are used in various fields such as health-care, biomedical science, drug and gene delivery, food industry, energy science, light emitters and catalysis [3-6]. The hybrid field of Bionanotechnology, that uses biological starting materials, biological design principles or has biological or medical applications, has found the vast variety of application due to the manipulation of living matter at the nanoscale [7, 8]. The synthesis of nanoparticles with different size, morphology and chemical composition is important area of nanobiotechnology research. This multidisciplinary approach results from the tentative use of nanoparticles in various disciplines such as biology, chemistry, biochemistry, medicine, physics and engineering. Furthermore, this hybrid fields also serves as imperious practice in developing clean, safe and environment friendly process for synthesizing metallic nanoparticles that reduce metals in a specific metabolic pathway [5, 6].

For nanoparticles synthesis, various approaches are applied comprising chemical, physical and biological methods (Figure 1). Traditionally applied methods (i.e. physical and chemical synthesis) have potential harmful effects such as environmental damage, cost and prolong time consumption; researchers are looking for other possible approaches for the synthesis of NPs [9]. Biological production of NPs has preference over other mentioned protocols as they are having more dynamic nature, comparatively safer and efficient

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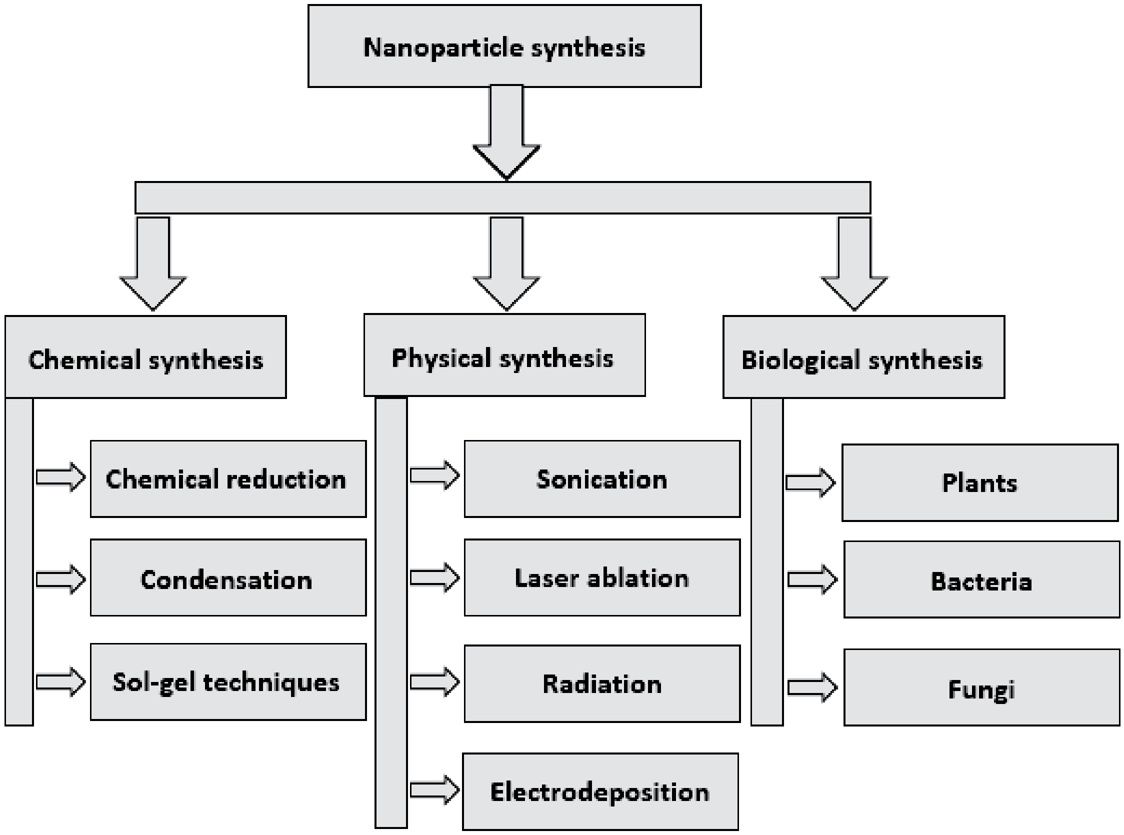


Fig. 1. Methods for NPs synthesis

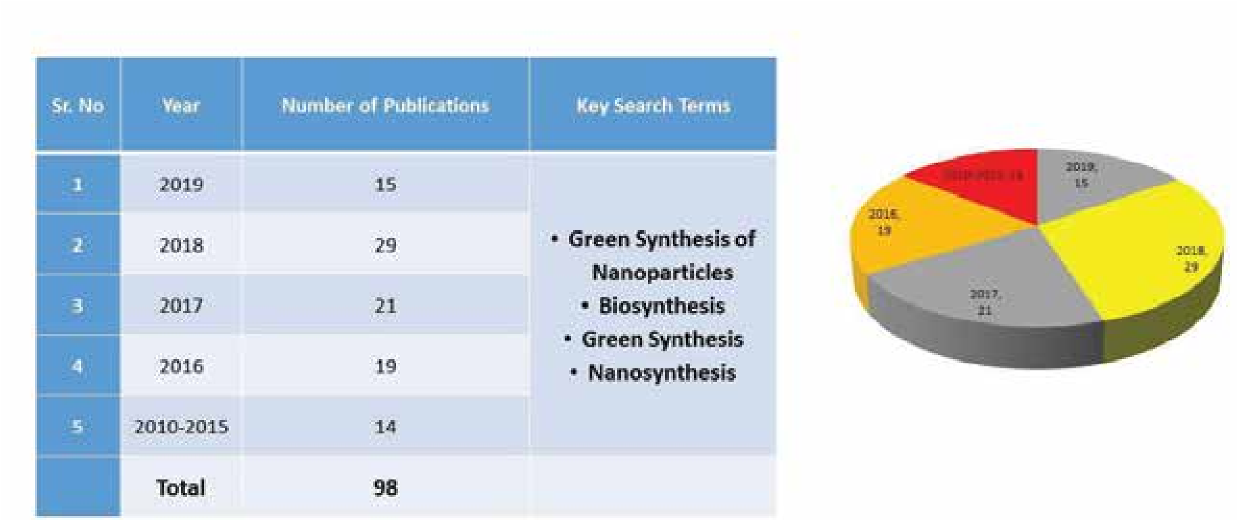
in terms of energy input [10, 11]. NPs are produced in *in-vivo* biological systems including eukaryotes and prokaryotes [12].

The development of ecofriendly process that avoids the use of toxic chemicals is the need of hour. Different techniques such as chemical, physical, biochemical and green synthesis are used for synthesis of nanoparticles. Green synthesis in particular, has been used on a large scale in Pakistan. Green synthesis approaches such as have tremendous advantages over chemical method that involved the use of potential toxic agents [7]. Among the chemical approaches, the use of inorganic reducing agents and radiolysis are extensively used for nanoparticles synthesis. Several chemicals methods are still in developmental stages because they face aggregation problems of nanoparticles, as well as growth control, morphology and size distribution. Moreover, nanoparticles extraction and purification for further application are still imperative issues [13].

In Pakistan, chemical methods have not been extensively used for the production of nanoparticles [14, 15]. In physical methods the most widely used are evaporation-condensation and laser ablation for synthesizing nanoparticles. The absence of solvent contamination, size uniformity and distribution are the advantages over chemical approach. Physical methods also withstand potential disadvantages such as large space requirement and high energy consumption [16, 17]. Non-biodegradable compounds produced by the widely used physiochemical methods, (i.e. photochemical reduction, UV irradiation and ultrasonic fields); are potentially hazardous to the environment and biological systems [17].

1.1. Biological Synthesis

Medicinal plants are predominantly utilized in green synthesis methods as they have potential advantages [18]. Researchers are screening plants for the purpose of identifying new compounds of medical importance [19]. Contribution of Pakistani



Sr. No	Year	Number of Publications	Key Search Terms
1	2019	15	• Green Synthesis of Nanoparticles • Biosynthesis • Green Synthesis • Nanosynthesis
2	2018	29	
3	2017	21	
4	2016	19	
5	2010-2015	14	
	Total	98	

Fig. 2. Number of publication in the line of Bioinspired synthesis of nanoparticles from 2010 to mid of 2019 (Source: Web of Science).

Table 1. Green synthesis of nanoparticles from various plants extracts. All these studies are performed in Pakistan.

References	**Plant species**	**Common name**	**Part used**	**Physical properties of NPs**		
				Shape	Characterization	Size (nm)
Tahir et al. [23]	*Taraxacum laevigatum*	Dandelion	---	Spherical	UV, XRD, TEM, SEM, EDX, DLS and FTIR	2–7
Zia et al. [24]	*Cydonia oblong*	Quince	Seed	Cubic	UV, FTIR, XRD and SEM	38
Tahir et al. [25]	*Salvadora persica*	Toothbrush tree	Stem	Spherical	UV, XRD, FTIR,HRTEM, SEM and EDX	1–6
Tahir et al. [26]	*Sapium sebiferum*	Vegetable Tallow	leaf	Spherical	UV, FTIR, XRD, HRTEM, SEM, TGA and DLS	5
khan el al. [27]	*Sueda fruciotosa*	Shrubby sea blight	Whole	Spheroid	UV, XRD, SEM HRTEM and FTIR	6-8
Ahmad et al. [28]	*Fagonia indica*	Dhreima	Whole	hexagonal	UV, SPR, DLS, HRTEM, XRD, FTIR and EDX	15-20
Javed et al. [29]	*Stevia rebaudiana Bertoni*	Candyleaf	Shoot		UV, XRD, FTIR	34
Zia et al. [30]	*Lycopersicon esculentum, Vitis vinifera*	Tomato, Grape	Fruit	Cubic	FTIR, XRD, SEM,	10-30
Phull et al. [31]	*Bergenia ciliata*	Zakham-e-Hayat	Whole	Spherical	UV, SEM, FTIR	35
Zia, F. et al. [24]	*Cydonia oblong*	Quince	Seed	Cubic	UV, FTIR, XRD	38
Khan et al. [32]	*Citrus sinensis var. Kozan yerly*	Citrus	Fruit	Spherical	UV, XRD, EDX, HRTEM and FTIR	4-10
Ullah, I. et al. [33]	*Teucrium stocksianum Boiss.*	Togreyern	Shoot	Cubical	UV, XRD, SEM, DLS and FTIR	< 100
Hameed, S. et al. [34]	*Silybum marianum*	Milk thistle	Shoot	Plate and Spherical	UV, HR-SEM, HR-TEM and FTIR	~26-27

Abbasi, B. H. et al. [35]	*Cannabis sativa*	Marijuana	Leaf	Face centered cubic	UV, XRD, FTIR, EDX and SEM	38.94 [29], 45.3 (Ag)
Nazir, S. et al. [36]	*Silybum marianum*	Milk thistle	Seed	Spherical, Triangular	XRD, FTIR, SEM and EDX	64-70 (C), 63-65 (WPE)
Hassan, D. et al. [37]	*Callistemon viminalis*	Bottlebrush	Flower	Spherical	UV, HR-SEM HR-TEM, XRD, FTIR and EDS	32, 26, 22
Khalil, A. T. et al. [38]	*Sageretia thea*	Bird plum	leaf	Face centered cubic	XRD, FTIR, Raman, EDS, SAED, HR-SEM and HR-TEM	~ 27
Afridi, M. S. et al. [39]	*Verbena officinalis, Verbena tenuisecta*	Verbena, Moss verbena	leaf	Rod, Flower shaped	UV, FTIR, XRD, SEM and TEM	65–75, 14–31
Anjum, S. et al. [40]	*Linum usitatissimum L.*	Linseed	Seed	Face-centered cubic	UV, XRD, FTIR, SEM and EDX	19–24
Hassan, D. et al. [41]	*Callistemon viminalis*	Bottle Brush	Flower	Rhombo-hedral	UV, XRD, FTIR, HR/SEM, TEM, EDX and SQUID	15, 17
Anjum, S. et al. [42]	*Phlomis bracteosa*	Jerusalem sage	Seed	Face-centered cubic	UV, FTIR, XRD, SEM and EDX	22.41
Khalil, A. T. et al. [43]	*Sageretia thea*	Bird plum	leaf	Face-centered cubic	XRD, HR-SEM, HR-TEM, EDS and ATR-FTIR	20.03
Khalil, A. T. et al. [44]	*Sageretia thea*	Bird plum	Leaf	Tetragonal	XRD, FTIR, Raman, EDS, HR-SEM/TEM and SAED	~30
Riaz, H. R. et al. [45]	*Catharanthusroseus-var. Alba*	Rosy periwinkle	Calli	Triangular	XRD, FTIR and SEM	77–79
Nasar, M. Q. et al. [46]	*Ephedra Procera C. A. Mey.*	Pinellia	Shoot	Spherical	UV, FTIR, XRD and SEM	17.2
Khalil, A. T. et al. [47]	*Sageretia thea*	Bird plum	Leaf	Hexagonal	XRD, FTIR, HR-SEM/TEM, EDS, Raman, SAED and UV	12.4
Khalil, A. T. et al. [48]	*Sageretia thea*	Bird plum	Leaf	Spherical	XRD, FTIR, HR-SEM/TEM, ATR- EDS, SAED and Raman	~18
Ovais, M. et al. [49]	*Olax nana Wall. ex Benth.*	Conchidium	Whole	Spherical/nanorods	XRD, FTIR, SEM, TEM, DLS, EDX, and SAED	26 (Ag), 47 [29]
Ullah, I. et al. [50]	*Teucrium stocksianum*	Mekhzani	Shoot	Face-centered cubic	UV, XRD	10–15 (Che), 10–40 (Bio)
Shinwari, Z. K. et al. [51]	*Sageretia thea*	Bird plum	Leaf	Hexagonal	XRD, TGA/DSC, FTIR, HR-TEM and Raman	28.09 ± 5

Abbreviations: UV; Ultra violet, EDS; Energy-dispersive X-ray spectroscopy, SPR; surface plasmon resonance, SAED; selected area (electron) diffraction, FESEM; field emission scanning electron microscope, SEM; scanning electron microscopy, TEM; Transmission electron microscopy, FTIR; Fourier Transform Infrared Spectroscopy, NMR; Nuclear Magnetic Resonance, XRD; X-Ray Diffractometer, AFM; atomic-force microscopy, XPS; X-ray photoelectron spectroscopy, TGA; thermogravimetric analysis, FFT; fast fourier transform, WPE; Whole plant extract, Bio; Biological method, Che; Chemical method.

researchers in the field of bioinspired synthesis of nanoparticles has been illustrated in Table 1 and Figure 2. Redox potential of phytochemicals confer the antioxidant activity in plant extracts [20], due to which plants perform vital activities such as neutralizing the free radicals and oxygen quenching. The higher antioxidant potential of nanoparticles is assumed to be due to nanoparticles that has antioxidant material from the plant extract upon its surface. Particle size, surface area and surface reactivity are the factors that determine the toxicity of the nanoparticles [21].

2. APPLICATIONS OF NANOPARTICLES

Nanotechnology has many applications in the field of medicines, drugs delivery, drugs analysis, catalytic activity, waste water treatment, cancer treatment, antimicrobial, antibacterial, and antifungal activities. Latest studies revealed that AuNPs are used to achieve functional electric coating. AuNPs are best biosensors, used for diagnosing of medicinal problems inside the body. Nanoparticles are now used as antiviral against HIV, Hepatitis-B, monkey pox virus and many other viral diseases. Nanosilver can act as viricidal agent by inhibiting the initial stages of HIV-I cycle [22].

2.1. Antibacterial Activities of NPs Reported from Pakistan

In Pakistan, various studies have been conducted confirming antibacterial activities of Biologically synthesized nanoparticles. *V. vinifera and L. esculentum* derived AgNPs were used against *S. typhi, E. aerogenes*, *M. luteus*, *P. septica, S. aureus* and *B. subtilis* for their antibacterial assessment. It revealed its highest Zone of Inhibition (ZOI) of 26 ± 1 mm against *S. aureus* [30]. *Bergenia ciliate* pharmacological assessment revealed broad spectrum antibacterial potential against multiple bacterial species [31]. AgNPs of *D. mucronata* revealed prominant antibacterial potency against *E. coli* and good it showed good potency against *P. vulgaris, P. aeruginosa, S. aureus, M. morganii*, *A. baumannii* and *VRSA* [52]. PtNPs of *Taraxacum laevigatum* showed substantial antibacterial potency against *B. Subtilis* and *P. aeruginosa* [23]. Palladium NPs (PdNPs) of *Sapium sebiferum* revealed substantial antibacterial activity against *B. subtilis* and *S. aureus*. The ZOI were reported as 29 (± 0.8 mm) and 19 (± 0.6 mm), while the said NPs exhibited moderate potency against *pseudomonas aeruginosa* [26]. AgNPs of tomato and grape juice exhibited significant bactericidal activity against *Staphylococcus aureus, Pseudomonas septica*, *Bacillus subtilis, Micrococcus luteus*, *Enterobacter aerogenes and Salmonella typhi* [30]. The most and least effective antibacterial potential of *Silybum marianum* mediated ZnO–NPs was reported against *B. Subtilis* and *P. aeruginosa*, while the effect of Ag–ZnO heterostructures against *B. subtilis* was similar and comparatively higher against *P. aeruginosa* [34]. *Cannabis sativa* leaf extract mediated bimetallic NPs used against five bacterial species exhibit bactericidal potency [35]. ZnO of *Silybum marianum'*s callus extract used against *Klebsiella pneumonia* and *Bacillus subtilus* through well- diffusion method exhibited antibacterial potential of 13 mm and 15 mm respectively against the tested bacterial strains. The aforementioned results of the ZnO were somewhat similar to the ZOI of the standard antibiotic Cefixime [36]. Iron oxide nanoparticles (IONPs) of *Callistemon viminalis* floral extract were used against nine gram negative and three gram positive bacterial strains for its bactericidal assessment at various concentrations through microplate-based technique that reports percent inhibition of bacterial strains. IONPs exhibited maximum antibacterial potential against *S. aureus, S. typhi, S. enterica* and *K. pneumonia* and exhibited least antibacterial potential against *S. dysenteriae* among all tested bacterial strains [37]. PbO NPs of *Sageretia thea* on its bactericidal potential assessment against Staphylococcus epidermis, *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* revealed maximum antibacterial potential against *Klebsiella pneumonia* among the tested bacterial strains with a MIC of 31.25 µg/mL and minimum antibacterial potential against *Pseudomonas aeruginosa* with a MIC of 250 µg/mL [38]. Callus extract (CE) mediated AgNPs of *Linum usitatissimum* L. were used for its antibacterial assessment against potent multi drug resistant species (*S. aureus, K. pneumoniae* and *E. coli*). The antibacterial potential of CE based NPs was found more than the NPs synthesized from whole plant extract [40], while AgNPs of *Phlomis bracteosa* exhibited 11.1±0.10, 10.3±0.11 and 13.2±0.12 mm ZOI against the aforementioned three pathogenic bacterial strains. They reported that antibacterial potency of the AgNPs of the *Phlomis bracteosa* was almost similar with that of Ampiclox (Standard antibiotic) [42]. Chromium oxide NPs (Cr_2O_3 NPs) of *Callistemon viminalis* exhibited dose dependent bactericidal response against the tested bacterial strains in the microplate method. *K. pneumoniae, P. vulgaris, C. sakazakii* and *V. cholera* were found more susceptible to Cr2O3 NPs with MIC of 12.5µg/ml and two bacterial strains (*Citrobacter, S. Aureus*) were found less susceptible to Cr2O3 NPs with a MIC 50 µg/ml [41]. Cobalt oxide (Co3O4) NPs of *Sageretia thea* was used against six bacterial strains, that comprised three gram negative and three gram positive bacteria. The NPs exhibited more lethality against *E. coli* and *S. aureus* while the least lethality was recorded against *Pseudomonas aeruginosa* [43]. *Sageretia thea* mediated IONPs were used against five bacterial strains at various concentrations ranging from 1000–31.25 µg/ml. Maximum activity of biogenic IONPs was reported against *S. epidermidis* and *P. aeruginosa* with 7.8 µg/ml MIC for both of the strains [44]. *Ephedra Procera* mediated AGNPs on its antibacterial assessment revealed MIC of 11.33 µg/ml and 11.12 µg/ml against *E. coli* and *B. subtilis* respectively that was highest reported activity while against *P. aeruginosa,* moderate activity was observed. *S. aureus* and *S. epidermidis* were resistant to the EPNPs [46]. ZnONPs of *Sageretia thea* exhibited maximum and minimum activity against *K. pneumonia* and *P. aeruginosa* respectively that were used in different concentration ranging from 2000 to 62.5 µg/ml [46]. Nickle oxide (NiO) NPs of *Sageretia thea* showed its maximum efficacy against *Bacillus subtilis* and *E. coli*. The reported ZOI were 15.1 mm and 14.1 mm respectively against the said pathogens. The least efficacy was reported against *K. pneumonia* and *P. Aeruginosa* [48]. Silver and gold NPs of *Olax nana* were used against four bacterial strains at 62.5–2000µg/ml concentration range. AgNPs revealed strong antibacterial potency against *Staphylococcus epidermidis* and *Escherichia coli* with MICs of 7.14µg/ml and 8.25µg/ml respectively, while AuNPs were found active only against *Staphylococcus aureus* with MIC of 9.14µg/ml [49]. CE-mediated ZnONPs of *Catharanthus roseus* produced, ZOI of 7 ± 1.25 mm for *E. coli* and 13 ± 0.7 mm for *B. subtilis* and no ZOI was recorded against *P. aeruginosa.* Melatonin and NAA stimulated CE-mediated ZnONPs were more active against *E. coli, P. aeruginosa* and *B. subtilis* with ZOI of 10 ± 0.57, 13 ± 0.54 mm and 17 ± 0.76 mm, respectively [45]. The above discussion suggests antibacterial properties of several biosynthesized nanoparticles however; there is no commercialized product in the country-Pakistan. As these products have potentials to address the emerging issue of antibiotics resistance therefore, it can be developed and marketed.

2.2. Antifungal Activities of NPs from Pakistan

Antifungal assessment of *Bergenia ciliate'*s NPs revealed antifungal potential against various fungal species [31]. AgNPs of *D. mucronata* leaves were used for its antifungal assessment and it only showed its potency against *C. albicans* and *A. niger* [52]. *Silybum marianum*-mediated ZnO–NPs and Ag–ZnO heterostructures, used against four fungal pathogenic strains at a concentration of 1000–50µg/ml were considered insignificant in comparison with standard antibiotics [34]. The Iron oxide-NPs of *Sageretia thea* exhibited highest percent inhibition of 79.03% ± 2.90, 74.58% ± 3.15 and 74% ± 3.20 against *R. solanai* followed by *A. fumigatus* and *M. racemosus* at a dosage of 2 mg/ml [44]. Antifungal assessment of *Ephedra Procera*'s NPs revealed considerable potential against *A. flavus* and *A. niger* while moderate activity against *Mucor spp* [46]. ZnONPs of *Sageretia thea* were used against five fungal strains that revealed linear growth inhibition against all tested fungal strains. *M. racemosus* and *A. fumigatus* were inhibited at all tested concentrations of the said NPs [47]. NiO NPs of *Sageretia thea* exhibited maximum fungicidal potential against *M. racemosus* and *R. solani* with percent inhibition of 64% and 63.2% respectively at 2 mg/ml concentration while it exhibited minimum fungicidal potential against *A. flavus* [48]. Future research could be directed towards commercial production of nanoparticle-based antifungal agents that can replace or complement conventional pharmaceutics in Pakistan.

2.3. Antioxidant Activities of NPs Reported from Pakistan

Plants contain multiple chemical constituents that have extensively studied to find their pharmacological importance. Among these constituents, major chemical groups such as flavonoids and polyphenols are reported as reducing agents. These chemicals have antioxidant potentials through which plant protect itself from oxidative stress. During NPs synthesis, the said chemical groups play important role in Ag +ions reduction [53]. Free radical scavenging potential of plant extracts and its derived AgNPs has been reported in various studies. AgNPs synthesized from the fruit extract of *Citrus sinensis var. Kozan yerly* has a good antioxidant potential, assessed through 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [32].

The development of reducing power is considered as causative reason for the antioxidant potential and this phenomenon has been demonstrated in various studies [54]. AgNPs are supposed to be electron donors, thus convert the free radicals to more stable form through donation of electrons [32]. AgNPs synthesized from tomato showed 76% and 83% free radical inhibition at 20 and 30 mM concentration. NPs synthesized from grapes were also significant for the said property. AgNPs of *Bergenia ciliata* were synthesized that showed 59.31% free radical scavenging potential in DPPH assay. The antioxidant potential (60.48±2.2) ascorbic acid equivalent (AAE) was reported of *B. ciliate* AgNPs [31]. *Daphne mucronata* mediated AgNPs exhibited its highest antioxidant potential of (85.4% AAE) at 600 µg/ml concentration [52]. AgNPs of *Lycopersicon esculentum* fruit juice exhibited 83% free radical scavenging potential at 30 mM concentration of $AgNO_3$ while the AgNPs of *Vitis vinifera* also exhibited prominent potential. The highest antioxidant potential of *Lycopersicon esculentum* fruit juice mediated AgNPs was 5.8 µg AAE/100 µg and that of *Vitis vinifera* was 8.3 µg AAE/100 µg, respectively [30]. *Silybum marianum* mediated ZnO and Ag–ZnO revealed highest antioxidant potential of 67.6±1.44 and 72.6±1.32 AAE respectively at 1000µg/ml concentration [34]. IONPs of *Callistemon viminalis* floral extract revealed its maximum free radical trapping potential of (55.16 ± 1.34% AAE) at the concentration of at 200mg/mL. The dose dependent antioxidant activity increased with an increase in the IONPs concentration and in antioxidant activities, analogous pattern was reported. The maximum power of reduction (51 ± 2.4% AAE) was reported at 200mg/mL concentration of IONPs [37]. PbO NPs of *Sageretia thea* exhibited 58 % AAE free radical trapping potential in DPPH assay. The scavenging potential and NPs concentration were found in direct relation. At 200 µg/ml, Moderate reducing power and moderate antioxidant capacity of 22 µg AAE/mg and 19.6 µg AAE/mg were reported respectively [38]. Co_3O_4 NPs of *Sageretia thea* exhibited 57 % free radical trapping potential in DPPH assay at 200 µg/ml concentration. At 200 µg/ml, maximum total reduction potential and total antioxidant potential of 19.8 and 23.6 µg AAE/mg was reported respectively [43]. The highest total antioxidant potential (38.21 ± 1.93 AAE) of *C. viminalis*'s α-Cr_2O_3 NPs was reported at 200µg/ml. The quenching ability of the said plant can be associated with phenolic compounds present in *C. viminalis* that might be involved in the capping of the α-Cr_2O_3 NPs. Maximum DPPH scavenging potential of 57.69 ± 2.19 AAE was reported at the concentration of 200µg/ml [41]. The highest total antioxidants potential of *Sageretia thea*'s ZnONPs was 25.6 ± 1.54 AAE/ milligram for the tested concentration of 200 µg/ml. In DPPH assay moderate radical scavenging (63.5 ± 2.4) was observed at 200 µg/ml and minimum scavenging was reported at 25 µg/ml [47]. NiO NPs of *Sageretia thea* revealed 65% to 3.27% DPPH radical quenching potency at a concentration range of 200lg/ml to 1lg/ml and 11.3lg AAE/mg antioxidant potential was observed at 200lg/ml [48]. These studies suggest that bioinspired NPs have potential for antioxidant activities that can be manipulated for formulation of different commercial products.

2.4. Cytotoxic Activities Assessed for NPs Reported from Pakistan

Daphne mucronata mediated AgNPs revealed a higher cytotoxicity of 73.33% and LD^{50} of 5.074 µg/ml [52]. AgNPs of *Bergenia ciliate* revealed enhanced cytotoxicity with a LD50 of 33.92µg/ml in brine shrimp lethality assay. The cytotoxic behavior of NPs can be associated with anticancer potential [31]. Cytotoxic potential of *Silybum marianum* mediated ZnO–NPs and Ag–ZnO heterostructures used against *Artemia salina* larvae revealed its highest activity of 80% and 70% (at 1000 µg/ml), respectively [34]. AgNPs of *Teucrium stocksianum* leaf extract revealed cytotoxicity against J774 macrophage cells. The NPs of the aforementioned plant extract successfully inhibited cells propagation with inhibitory concentration (IC50) of 110.98 µg/ml [33]. The PbO of *Sageretia* *thea* exhibited dose dependent cytotoxic response against *Artemia salina* (Brine shrimp) with the median lethal dosage 27.74 µg/ml [38] while the median lethal dosage of cobalt oxide NPs of the said against the said species (Brine shrimp) was reported 19.18 µg/ml [43]. IONPs of *Sageretia thea* revealed mortality of 100% against brine shrimps at 200 µg/ml concentration [44]. *Ephedra procera* mediated AgNPs on its cytotoxic assessment revealed cytotoxic potential against HepG2 Cells with 61.3 and 247µg/ml as inhibitory concentration (Median) [46]. Zinc oxide nanoparticles (ZnO NPs) of *Sageretia thea* revealed its IC_{50} as 21.29µg/ml with 80% cytotoxic response against *Artemia salina* [47]. Nickel oxide nanoparticles (NiO NPs) of *Sageretia thea* has shown IC50 value as 42.60l g/ml against brine shrimp [48]. Silver nanoparticles (AgNPs) of *Teucrium stocksianum* were assessed through MTT assay that revealed 80% growth inhibition at 266µg/ml [50]. Further research can be directed towards understanding of molecular pathways (of different NPs) that are involved in the mechanism of NPs-mediated cytotoxicities. This will help to differentiate the products/NPs that have anticancer potential.

2.5. Anticancer Activities of NPs Reported from Pakistan

AgNPs of *Teucrium stocksianum*'s aqueous extract revealed dose dependent upsurge in its anti-oncogenic potential against Michigan Cancer Foundation-7 (MCF-7), a breast cancer cell line. Additional killing of the aforementioned cancer cell line was observed when the cell line was exposed for longer duration [33]. Bioinspired IONPs of *Callistemon viminalis* used against HepG2 cells for its cytotoxic assessment through MTT assay in multiple concentrations ranging from 500 to 7.8mg/ml. IONPs exhibited substantial cytotoxicity against the said cell line with 80% mortality of oncogenic cells at 500mg/ml. The anticancer potential of the NPs reported was in direct relation with the concentration of the NPs as the activity increased with increase in the concentration of *Callistemon viminalis*'s NPs [37]. *Callistemon viminalis* mediated α-Cr2O3 NPs used for the assessment of its anticancer activity against HepG2 revealed dose dependent inhibitory potency with a lethal concentration (median) of 46.32µg/ml. The results revealed that the said NPs were found effective even with a low dosage of 7.8µg/ml [41]. *Olax nana*'s silver and gold NPs were used against HepG2 cell line with multiple dose concentrations (3.9–500µg/ml). Preferential dose dependent cytotoxic potency of the NPs was revealed. IC_{50} values of AuNPs was 2.97 µg/ml, followed by AgNPs (14.93 µg/ml). IC50 value of crude extract of the said plant was > 200µg/ml [49]. Bioinspired-NPs could be developed as a low cost therapeutic option against different kinds of cancers. However, it needs further research and experimental trials.

2.6. Photo Catalytic Activities of NPs Reported from Pakistan

The living environment sustains damage from industries that leave untreated hazardous soluble organic and inorganic pollutants. Synthetic dyes are considered more toxic for living organism due to their carcinogenic potential [55]. Methylene blue (MB) is used in textile industries can cause a list of disorders such as skin irritation, eye burns and gastrointestinal complication and many more. Photo degradation is extensively used waste water processing [56, 57].

Multiple approaches are employed for organic dyes removal from polluted water [58, 59] but they sustain limitations such as high cost and energy consumption [60]. Biologically synthesized AgNPs using *Salvadora persica* stem extract was used as a photo degradative tool for MB as the method is comparatively energy and cost effective. The photo degradation of MB by AgNPs in the presence of light was reported by the sharped decline in the absorption peak compare to controlled sample [25]. PdNPs of *Sapium sebiferum* leaf extract exhibited prominent outcome in the decomposition of MB. Significant photocatalytic potency of 90% was reported after 70 minutes at a concentration of 10 ml [26], while the Gold NPs (AuNPs) of *Fagonia indica* revealed 80% photocatalytic activity of the said chemical in a time span of 80 minutes. The AuNPs also exhibit photocatalytic activity against an organic pollutant, nitro phenol [28]. *Sueda fruciotosa* AuNPs also exhibit significant photocatalytic potency against MB [27]. As most of the commercial dyes from industries are left untreated that pollutes our environment. Research should be directed towards NPs-mediated treatment of these pollutants on mass scale.

3. CONCLUSION

The applications of nanotechnology are extended to multiple scientific fields; ranging from drug development, drug delivery, gene delivery, diagnostics and energy and other fields. These nanostructures have triggered a significant attention towards its use due to their unique characteristics such as small size and shape that make these nanoparticles competent for a wide range of medical and pharmacological applications. There are several reports from Pakistan on the multidisciplinary basics concepts of biosynthesized NPs however; there is a tremendous potential for commercial production of these NPs-based products.

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