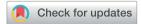
# **RSC Advances**



### **REVIEW**

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# Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: a review of recent literature

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Synthesis of metal nanoparticles using plant extracts is one of the most simple, convenient, economical, and environmentally friendly methods that mitigate the involvement of toxic chemicals. Hence, in recent years, several eco-friendly processes for the rapid synthesis of silver nanoparticles have been reported using aqueous extracts of plant parts such as the leaf, bark, roots, etc. This review summarizes and elaborates the new findings in this research domain of the green synthesis of silver nanoparticles (AgNPs) using different plant extracts and their potential applications as antimicrobial agents covering the literature since 2015. While highlighting the recently used different plants for the synthesis of highly efficient antimicrobial green AgNPs, we aim to provide a systematic in-depth discussion on the possible influence of the phytochemicals and their concentrations in the plants extracts, extraction solvent, and extraction temperature, as well as reaction temperature, pH, reaction time, and concentration of precursor on the size, shape and stability of the produced AgNPs. Exhaustive details of the plausible mechanism of the interaction of AgNPs with the cell wall of microbes, leading to cell death, and high antimicrobial activities have also been elaborated. The shape and size-dependent antimicrobial activities of the biogenic AgNPs and the enhanced antimicrobial activities by synergetic interaction of AgNPs with known commercial antibiotic drugs have also been comprehensively detailed.

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#### Introduction

Nanotechnology is gaining enormous attention as a new area of research dealing with the development of nanomaterials and nanoparticles (NPs) for their utilization in diverse fields such as catalysis, electrochemistry, biomedicines, pharmaceuticals, sensors, food technology, cosmetics, *etc.*<sup>1-3</sup> Nanoparticles (NPs) are nanometer-sized (<100 nm) atomic or molecular scale solid particles having some excellent physical properties compared to the bulk molecules depending on their size and morphology. Among all types of NPs, metal and metal oxide nanoparticles have been thoroughly examined using science and technology due to their excellent properties such as high surface to volume ratio, high dispersion in solution, *etc.*<sup>6,7</sup> Owing to these, metal

Currently, modified or fabricated of NPs is widely utilized in industrially manufactured items *e.g.*, cosmetics, electronics, and textiles. Furthermore, the rapid increased in the number of microbes resistant to existing antibiotic drugs that has led to the requirement of novel medicines in the form of bare NPs or in conjunction with existing antibiotics to exert a favourable synergistic effect resulted in the wide spread use of NPs in several medical fields.<sup>10,11</sup> Nowadays, NPs have been utilized for molecular imaging to achieve profoundly resolved pictures for diagnosis. In addition, contrast agents are impregnated onto NPs for the tumour and atherosclerosis diagnosis.<sup>12–14</sup> Furthermore, nanotherapeutic has been promoted everywhere throughout the world after the first FDA affirmed nanotherapeutic in 1990, to build up different nano-based drugs.<sup>15</sup>

At the beginning of 20<sup>th</sup> century, various physical and chemical methodologies such as chemical reduction, milling *etc.*, have been utilized for the synthesis of NPs synthesis as well as to enhance its efficiency. However, these conventional techniques involve costly and toxic chemicals and cannot be considered an environmentally benign process. Taking into account, nowadays researchers showed great interest on the synthesis of metal and metal oxides NPs employing bio-genic route, that utilized aqueous plant extract and microbes, as

and metal oxide nanoparticles display enhanced antimicrobial properties.<sup>8,9</sup>

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they are environment-friendly, stable, clinically adaptable, biocompatible and cost-effective. Therefore, bio-inspired technology for NPs synthesis became a significant branch in the field of nanoscience and nanotechnology. In Till now, numerous metal and metal oxide NPs have been synthesized using plant extract and microbes etc. NPs have been synthesized availability, renewability and environment-friendly nature, in addition to their vast applications in the synthesis of NPs, plant biomass are also largely targeted by our group and others as a catalyst for chemical synthesis And biodiesel productions.

Among metal NPs, silver NPs is gaining enormous interest in the research community due to their wide scope of application in microbiology, chemistry, food technology, cell biology, pharmacology and parasitology.<sup>27,28</sup> The morphology of the silver NPs is the deciding factor of their physical and chemical properties.28 Basically, several techniques such as sol-gel method, hydrothermal method, chemical vapour deposition, decomposition, microwave-assisted combustion method etc., have been utilized for the synthesis of silver NPs.<sup>29-31</sup> Recently, bio-genic synthesis of silver NPs (AgNPs) using biomaterials such as plant extract and microbes as reducing agent and their antimicrobial activity is widely investigated.<sup>32,33</sup> AgNPs are produced by oxidation of Ag<sup>+</sup> to Ag<sup>0</sup> by different biomolecules such as flavonoids, ketones, aldehydes, tannins, carboxylic acids, phenolic and the protein of the plant extracts.

UV-visible spectroscopy is a simple and widely used analytical technique to monitor the formation of AgNPs. Upon interaction with an electromagnetic field, the conducting electrons present in the outermost orbital of metal NPs collectively oscillate in resonance with certain wavelengths to exhibit a phenomenon called surface plasmon resonance (SPR). The excitation of SPR is responsible for the formation of color and absorbance in a colloidal solution of AgNPs. The SPR peaks at around 435 nm are usually taken to confirm the reduction of silver nitrate into AgNPs. The general, spherical NPs exhibit only a single SPR band in the absorbance spectra, whereas two or more SPR bands were observed for anisotropic particles depending on the shape. The absence of peak in the region 335 and 560 nm in UV-Vis spectra are sometime used as an indication of the absence of aggregation in NPs. 32,36

Statistical data analysis in Fig. 1 depicted the increasing trend of published research papers in the field of biogenic

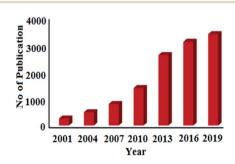


Fig. 1 Publications per year for green synthesis of AgNPs during the period 2001 to 2019 (data collected from SciFinder Database).

synthesis of AgNPs. These data were collected in September 2020 from "SciFinder Database" using the keyword "Green synthesis of silver nanoparticles". From a meagre 259 publications in the year 2001, it has exponentially increased to 3374 publications in 2019. Thus, in this review, an attempt has been made to inspire the researchers to explore the natural resources to synthesize silver nanoparticles by diverse plants and their organs to interconnect nanotechnology with biotechnology into one, termed as nanobiotechnology. This review will also unlock ideas to utilize different paths for the production of silver nanoparticles, which can help human beings. We have comprehensively discussed the bio-genic synthesis and silver nanoparticles using various plants and their application in antimicrobial activity. We also discussed the effect of the synthesized silver nanoparticles' size and shape in antimicrobial activity towards various pathogenic bacteria. In an attempt to synthesize metals NPs one has to bear in mind that the success of NPs depends not only on the size and shape but also on stability of NPs as they have the tendency to form large aggregates that lead to precipitation, thereby reducing their efficacy.

# 2. Protocols for the biosynthesis of AgNPs

Biogenic synthesis of AgNPs is an easy single-step protocol without generating harsh and toxic chemicals; hence, they are save, economical and eco-friendly. In recent years, both plant and microbes are extensively investigated for the biosynthesis of AgNPs of varying size, shape, stability, and antimicrobial efficacy.

#### 2.1 From plant extract

Various parts of plant such as leaves, roots, flowers, fruits, rhizomes etc., have been successfully utilized for the synthesis of AgNPs. 37-39 Different parts of plant are collected from various sources, washed properly with ordinary water followed by distilled water to exclude debris and any other unwanted materials. After that, the portions are dried and ground to make powder or utilized as fresh to make the extract. To prepare the extract, the chopped pieces or the ground powder of the parts of the plant are put in deionized water or alcohol and usually heated below 60 °C for few hours as high-temperature heating long time may leads to the decomposition of phytochemicals in the biomass extract. Plant extract of different pH is added to the solutions having a different concentration of Ag salt as metal precursor followed by heating at different temperature led to the synthesis of AgNPs.40-42 This synthesis process avoids the use of chemical stabilizer as biomaterials present in the extract act as a reducing agent as well as a stabilizing agent for the synthesis of AgNPs. 43,44 The progress of the formation of AgNPs can be monitored by visual color changes or using UV-Vis. Spectroscopy, where a sharp peak due to surface plasmon resonance (SPR) of AgNPs at around 430-450 nm is clearly observed.34 After successful synthesis of the AgNPs, the mixture is centrifuged at high rpm to separate the NPs followed by **RSC Advances** Review

proper washing using solvents and dried in an oven at low temperature. 45,46 The different plant parts extracts that have been successfully utilized in the green synthesis of AgNPs are given in Fig. 2.

#### 2.2 From microbes

Nowadays, the use of microbial cell for the synthesis of metal NPs has come out as a great approach. Microbial cells turn to be excellent biofactories for the synthesis of AgNPs. 47,48 At first, the cultures are allowed to develop as culture suspension in disinfected distilled water having the culture medium. Then, different concentration of precursor of AgNPs is added into the cultured microbial followed by continuous mechanical stirring under dark conditions. The progress of the reaction is monitored by UV-Vis spectrophotometer. Finally, the resultant AgNPs is separated from the mixture via centrifugation at around 3000 rpm for 10-15 min.49

# Plant-mediated biogenic synthesis of AgNPs and their antimicrobial activity

Owing to the environmental issue, biogenic synthesis of metal and metal oxide NPs is gaining immense attention from the past decades. Reported literature revealed that various plant parts such as leaf, roots, seed, fruits and stem etc., have been utilized for the biosynthesis of NPs. The synthesis of NPs is fully dependent on the biomaterials/phytochemicals present in the extract. This section aims to discuss the various plant parts extract mediated synthesis of AgNPs and their application as antimicrobial.

#### 3.1 From leaf

To date, a numerous number of leaves extract have been utilized for the biosynthesis of AgNPs as shown in Table 1. Skimmia laureola was reported for the synthesis of spherical AgNPs with



Fig. 2 Different parts of plants used for biosynthesis of antimicrobial silver nanoparticles.

size 38  $\pm$  0.27 nm and tested against E. coli, K. pneumoniae, P. aeruginosa, P. vulgaris, S. aureus. 50 Miri et al. 51 have utilized Prosopis farcta extract for the biosynthesis of AgNPs with an average size of 10.8 nm at room temperature (RT). The antimicrobial activity of synthesized AgNPs was tested using disc diffusion method against the Gram-positive (Staphylococcus aureus (PTCC 1431), Bacillus subtilis (PTCC 1420)) and Gramnegative bacteria (Escherichia coli (PTCC 1399), Pseudomonas aeruginosa (PTCC 1074)) and compared with the control. The results showed that the inhibition diameter is increased for every tested pathogen (Fig. 3), indicates that synthesized AgNPs induces cellular damage to the bacteria's, hence can be used as nanoantibiotics. Aloe vera, 52 Eclipta alba, 53 Momordica charantia,54 Leptadenia reticulata55 are also used for the production of spherical biogenic AgNPs. In another study, AgNPs were synthesized by using tea leaf extract. Bactericidal activity of the synthesized NPs was tested against S. aureus and E. coli showed that inhibition action is more effective in case of S. aureus (89% inhibition rate) compared to E. coli (75% inhibition rate). In addition, treatment of the NPs against the bacteria leads to impairment of bacterial cell-cell adhesion.<sup>56</sup> Mukia maderaspatana leave extract was utilized for the biosynthesis of AgNPs with the size range of 58-458 nm. The synthesized nanoparticle was conjugated to the antibiotic ceftriaxone to investigate the antimicrobial activity towards the human pathogens such as B. subtilis, K. pneumonia, S. typhi, S. aureus and compared with the pathogen inhibition efficiency of the free nanoparticle and the antibiotic. The result obtained revealed that the AgNPs conjugated with ceftriaxone showed highest inhibition activity compared to the others.57

Clitoria ternatea and Solanum nigrum<sup>58</sup> were also reported to synthesize very small-size AgNPs and evaluated against B. subtilis, S. aureus, S. pyogenes, E. coli, P. aeruginosa, K. aerogenes. Interestingly, among the two leaf Clitoria ternatea extract gave smaller nanoparticles, which indicated the important role of extract constituents on the size of the produced nanoparticles. In addition, AgNPs of Clitoria ternatea showed higher activity than the AgNPs of Solanum nigrum against nosocomial pathogens due to its small size. It has been well-documented in literature that smaller size NPs showed higher antimicrobial activities due to larger surface area.<sup>56</sup> Grewia flaviscences,<sup>59</sup> Prunus yedoensis, 60 Justicia adhatoda L,61 Withania somnifera62 produced AgNPs in the range 8-100 nm which mainly are spherical. Numerous microbes such as skin bacteria are responsible for skin infection and body odor, as well as odor in feet, shoes, and/or socks mediated through the breakdown of amino acids present in sweat. Hence proper medication is required for human's wellbeing. Velmurugan et al. 60 applied the synthesized AgNPs from Prunus yedoensis to treat P. acnes, S. epidermidis, a well-known skin bacteria, and found that the synthesized NPs are more effective against skin bacteria than commercial AgNPs. The biogenic AgNPs showed 18 mm ZOI (zone of inhibition) in 30 μg scale against *P. acnes*, whereas commercial AgNPs displayed a lower ZOI of only 12 in the same

Pistacia atlantica,63 Tectona grandis Linn,64 Ficus virens65 also reported for the synthesis of AgNPs and are evaluated against

Table 1 Various leaf extract used for the green synthesis of AgNPs and their antimicrobial activity

No.	Plants	Size and shape	Test microorganisms	Ref
1	Skimmia laureola	Spherical; 38 $\pm$ 0.27 nm	E. coli, K. pneumoniae, P. aeruginosa, P. vulgaris, S. aureus	50
2	Prosopis farcta	Spherical; 8–11 nm	S. aureus, B. subtilis, E. coli, P. aeruginosa	51
3	Aloe vera	Spherical; 70 nm	Aspergillus sp., Rhizopus sp.	52
4	Eclipta alba	310 to 400 nm	E. coli, S. aureus, P. aeruginosa	53
5	Momordica charantia	Spherical; 11–16 nm	B. spp., S. spp., P. spp., E. coli, A. niger subsp., A. flavus subsp., P. spp.	54
6	Leptadenia reticulata	Spherical; 50–70 nm	S. pneumonia, K. pneumonia	55
7	Tea leaf	Spherical; 20 nm	S. aureus, E. coli	56
8	Raphanus sativus	Spherical; 6–38 nm	A. fumigatus, C. specifier, F. solani	40
9	Mukia maderaspatana	Spherical; 58–458 nm	B. subtilis, K. pneumonia, S. typhi, S. aureus	57
11	Clitoria ternatea	Spherical; 20 nm	B. subtilis, S. aureus, S. pyogenes, E. coli, P. aeruginosa, K. aerogenes	58
12	Solanum nigrum	Spherical; 28 nm	B. subtilis, S. aureus, S. pyogenes, E. coli, P. aeruginosa, K. aerogenes	58
13	Croton sparsiflorus morong	Spherical; 22–52 nm	S. aureus, E. coli, B. subtilis	46
14	Grewia flaviscences	Spherical; 60 nm	Bacillus, P. aeruginosa	59
15	Terminalia arjuna	Spherical; 8–16 nm	S. aureus, E. coli	21
16	Prunus yedoensis	Spherical, oval; 18–20 nm	<i>P. acnes</i> , <i>S. epidermidis</i> (skin bacteria)	60
17	Justicia adhatoda L.	Spherical; 5–50 nm	P. aeruginosa	61
18	Withania somnifera	70–110 nm; spherical	S. aureus, P. aeruginosa, C. albicans, P. vulgaris, E. coli, A. tumefaciens	62
19	Pistacia atlantica	Spherical; 10–50 nm	S. aureus	63
20	Tectona grandis Linn	Spherical; 26–28 nm	E. coli and S. aureus	64
21	Ficus virens	Spherical; 4.98–29 nm	B. subtilis, S. epidermidis, E. faecalis, K. pneumoniae, V. cholerae, V. vulnificus	65
22	Azadirachta indica	Spherical; 250–700 nm	E. coli	66
23	Artocarpus altilis	Spherical; 20–50 nm	E. coli, P. aeruginosa, S. aureus, A. versicolor	67
24	Crotalaria retusa	Spherical; 80 nm	E. coli and S. aureus	68
25	Cardiospermum halicacabum	Spherical; 74 nm	P. vulgaris, P. aeruginosa, S. aureus, B. subtilis, S. paratyphi, A. solani, F.	69
			oxysporum	
26	Psidium guajava	Spherical; 10–90 nm	P. aeruginosa	70
27	Cassia fistula	Spherical; 39.5 nm	B. subtilis, S. aureus, E. coli, P. aeruginosa, C. albicans, C. kruseii, C. viswanathii, T. mentagrophytes	71
28	Terminalia chebula	Spherical; 10–30 nm	E. coli, B. subtilis	72
29	Pedalium murex	Spherical; 20–50 nm	E. coli, K. pneumonia, M. flavus, P. aeruginosa, B. subtilis, B. pumilus and S. aureus	73
30	Azadirachta indica	Spherical; 34 nm	E. coli, S. aureus	74
31	Croton bonplandianum	Spherical; 15–40 nm	P. aeruginosa, E. coli, S. aureus	75
32	Tamarix gallica	Spherical; 5–40 nm	E. coli	76
33	Urtica dioica	Spherical; 20–30 nm	B. cereus, B. subtilis, S. aureus and S. epidermidis, E. coli, K. pneumoniae, S. marcescens, S.	77

Table 1 (Contd.)

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No.	Plants	Size and shape	Test microorganisms	Ref.
34	Ziziphus oenoplia	Spherical; 10 nm	P. aeruginosa, K. pneumoniae, E. coli, S. typhi	78
35	Lawsonia inermis	Spherical; 25 nm	E. coli, Pseudomonas spp., Bacillus spp., Staphylococcus spp., A. niger, A. flavus, Penicillium spp.	79
36	Lantana camara	Spherical; 20–200 nm	S. aureus, E. coli, P. aeruginosa, K. pneumonia	81
37	Jatropha curcas	Spherical; 20–50 nm	E. coli, P. aeruginosa, B. cereus, S. enterica, L. monocytogenes, S. aureus	82
38	Salvinia molesta	Spherical; 10 nm	S. aureus, E. coli	83
39	Sesbania grandiflora	Spherical; 20 nm	E. coli, Pseudomonas spp., Bacillus spp., Staphylococcus spp., A. niger subsp., A. flavus	84
40	Indoneesiella echioides	Spherical; 29 nm	subsp., <i>Penicillium</i> spp. R. rhodochrous, A. hydrophila, S. aureus, Pseudomonas aeruginosa, C. albicans	85
41	Phlomis	Spherical; 25 nm	S. aureus, B. cereus, S. typhimurium, E. coli	86
42	Hydrocotyle rotundifolia	Spherical; 7.39 nm	E. coli	87
43	Maclura pomifera	Spherical; 6–16 nm	S. aureus, Bacillus cereus, E. coli, P. aeruginosa, A. niger, C. albicans	88
44	Paederia foetida Linn.	Spherical; 5–25 nm	B. cereus, S. aureus, E. coli, A. niger	89
45	Atalantia monophylla	Spherical; 35 nm	B. subtilis, B. cereus, S. aureus, E. coli, P. aeruginosa, K. pneumoniae, C. albicans, A. niger	90
46	Talinum triangulare	Spherical; 13.86 nm	E. coli, S. typhi, B. subtilis, S. aureus, C. albicans	91
47	Ricinus communis	Spherical; 8.96 nm	S. aureus, P. aeruginosa	92
48	Erythrina suberosa	Spherical; 15–34 nm	S. aureus, P. aeruginosa, C. kruseii, T. mentagrophytes	93
49	Lippia citriodora	Spherical; 10–45 nm	S. aureus, B. subtilis, S. typhi, E. coli, C. albicans	94
50 51	Brassica oleracea L. Catharanthus roseus	Spherical; 30–100 nm Spherical; 10–88 nm	S. aureus, E. coli, C. albicans E. coli, C. koseri, K. pneumonia, P. aeruginosa, and S. aureus	95 96
52	Lavandula x intermedia	Spherical; 11–47 nm	E. coli, P. aeruginosa, P. mirabilis, B. cereus, K. oxytoca, S. typhi, S. aureus, C. albicans, A. niger, F. oxysporum	98
53	Canna edulis	Spherical; less than 40 nm	B. cereus, S. aureus, E. coli, S. typhimurium, E faecalis, C. tropicalis, C. kruseii, C. lusitaniae, C. guilliemondii, P. chrysogenum	99
54	Artemisia vulgaris	Spherical; 27–53 nm	E. coli, S. aurous, P. aeruginosa, K. pneumoniae, H. influenza	100
55	Psidium guajava	Spherical; 25 nm	B. aryabhattai, B. megaterium, B. subtilis, A. creatinolyticus, E. coli, Alcaligenes faecalis, S. cerevisiae, A. niger, R. oryzae	102
56	Taraxacum officinale	Spherical; 5–30 nm	X. axonopodis, P. syringae	104
57	Petiveria alliacea L.	Spherical; 16.7–33.74 nm	E. coli, K. pneumoniae, S. aureus	105

Table 1 (Contd.)

No.	Plants	Size and shape	Test microorganisms	Ref
58	Nervalia zeylanica	Spherical; 34.2 nm	S. aureus, L. brevis, P. putida, Pseudomonas sp., P.	106
	_, ,		chrysogenum, P. citrinum	
59	Ficus ingens	Spherical; 81.37 nm	E. coli, S. typhi, B. cereus	107
60	Thymbra spicata	Spherical; 70.2 nm	B. cereus, S. aureus, E. coli, S. typhimurium	108
61	Indigofera tinctoria	Spherical; 9–26 nm	B. pumilis, S. aureus,	110
		. ,	Pseudomonas sp., E. coli, A.	
			fumigatus, A. niger	
62	Tecoma stans	Spherical; 2–40 nm	B. subtilis, S. aureus, K. pneumoniae	114
63	Salvia leriifolia	Spherical; 27 nm	P. aeruginosa, E. coli, S. coagulase, C. frurdii, E. aerogenes, A. baumannii, S. marcescens, K. pneumonia, S.	115
			pneumoniae	
64	Leucaena leucocephala L.	Spherical; 25–50 nm	P. aeruginosa, S. pyogenes, S. aureus, E. coli, S. typhi, B. subtilis	116
65	Selaginella bryopteris	Spherical; 5–10 nm	S. aureus, E. coli, A. niger	117
66	Galega officinalis	Spherical; 27.12 nm	E. coli, P. syringae, S. aureus	118
67	Camellia sinensis	Spherical; 30 nm	S. aureus, K. pneumoniae	119
68	Justicia spicigera	Spherical; 86–100 nm	B. cereus, K. pneumoniae, and E. aerogenes, M. phaseolina, A. alternate, Colletotrichum sp., F. solani	120
69	Kleinia grandiflora	Spherical; 20–50 nm	P. aeruginosa, C. albicans	121
70	Eucalyptus citriodora	Spherical; 17.51 nm	C. albicans, A. baumannii, E. coli, K. pneumoniae, P. aeruginosa	122
71	Juniperus procera	Spherical and cubic; 30–90 nm	M. luteus, B. subtilis, P. mirabilis, K. pneumoniae, C. albicans	123
72	Capparis zeylanica	Spherical; 23 nm	S. epidermis, E. faecalis, S. paratyphi, S. dysenteriae, C. albicans, A. niger	124
73	Caesalpinia pulcherrima	Spherical; 9 nm	B. cereus, B. subtilis, S. aureus, C. rubrum, E. coli, P. aeruginosa, S. typhimurium, K. pneumoniae, C. albicans, C. glabrata, C. neoformans	126
74	Ligustrum lucidum	Spherical; 13 nm	S. turcica	127
75	Aesculus hippocastanum	Spherical; $50 \pm 5 \; \mathrm{nm}$	S. aureus, S. epidermidis, L. monocytogenes, C. renale, M. luteus, B. subtilis, B. cereus, E. faecalis, P. aeruginosa, P. fluorescens, E. coli, E. aerogenes, K. pneumonia, P. mirabilis, C. albicans, C. tropicalis, C. krusei	128
76	Melaleuca alternifolia	Spherical; 11.56 nm	S. aureus, methicillin- resistant Staphylococcus aureus, S. epidermidis, S. pyogenes, K. pneumoniae, P. aeruginosa, T. mentagrophytes, C. albicans	129
77	Carya illinoinensis	Spherical; 12–30 nm	E. coli, P. aeruginosa, S. aureus, L. monocytogenes	130
78	Murraya koenigii	Spherical;; 35–80 nm	E. coli, P. aeruginosa, E.	131
79	Clerodendrum inerme	Spherical; 5.54 nm	faecalis, C. albicans B. subtilis, S. aureus, Klebsiella, E. coli, A. niger, T. harzianum, A. flavus	132

Table 1 (Contd.)

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No.	Plants	Size and shape	Test microorganisms	Ref
80	Aspilia pluriseta	Spherical; 1–20 nm	B. subtilis, S. aureus, E. coli, P. aeruginosa, C. albicans	133
81	Melia azedarach	Spherical; 18 to 30 nm	V. dahliae	134
82	Scoparia dulcis	Spherical; 3–18 nm	P. aeruginosa, E. coli, B.	135
J_	Scopul in annous	•	subtilis, S. aureus, A. niger, C. albicans	100
83	Lantana trifolia	Spherical; 5 and 70 nm	E. coli, P. aeruginosa, C. albicans, S. aureus, B. subtilis	136
84	Mikania micrantha	Spherical; 10–20 nm	B. subtilis, E. coli, P. aeruginosa, S. pneumonia	137
85	Solanum nigrum	Spherical; 3.46 nm	E. coli	138
86	Curcuma longa L.	Spherical; 15–40 nm	S. aureus, P. aeruginosa, S. pyogenes, E. coli, C. albicans	139
87	Syzygium cumini	Spherical; 11–19 nm	S. aureus, A. flavus, A. parasiticus	140
88	Cleistanthus collinus	Spherical however not mentioned in manuscript; 30 to 50 nm	S. sonnei, P. aeruginosa, S. aureus, B. subtilis, S. dysenteriae, V. cholerae, P. mirabilis	142
89	Cestrum nocturnum	Spherical; 20 nm	Citrobacter, E. faecalis, S. typhi, E. coli, P. vulgaris and V. cholerae	143
90	Rice	Spherical; 16.5 nm	R. solani	144
91	Mentha aquatica	Spherical; 8 nm	P. aeruginosa, E. coli, B. cereus, and S. aureus	146
92	Rosmarinus officinalis	Sphere; 29 nm	S. aureus, B. subtilis, E. coli, P. aeruginosa	147
93	Ceropegia thwaitesii	Sphere; 100 nm	S. typhi, B. subtilis, S. aureus, S. epidermis, V. cholerae, S. epidermidis, K. pneumonia, M. luteus, P. mirabilis, P. aeruginosa, S. flexneri	148
94	Ziziphus jujuba	Irregular; 20–30 nm	E. coli	149
95	Ocimum tenuiflorum, Solanum trilobatum, Syzygium cumini, Centella asiatica and Citrus sinensis	Irregular; 28 nm, 26.5 nm, 65 nm, 22.3 nm and 28.4 nm	S. aureus, P. aeruginosa, E. coli, K. pneumoniae	154
96	Amaranthus gangeticus Linn	Globular-shaped; 11–15 nm	S. flexneri, B. subtilis, Sclerotinia sp.	155
97	Andrographis paniculata	Cubic; 40 and 60 nm	P. aeruginosa, E. coli, V. cholerae, S. flexneri, B. subtilis, S. aureus, M. luteus	156
98	Andrographis echioides	Cubic, pentagonal, hexagonal; 68.06–91.28 nm;	E. coli, S. aureus, S. typhimurium, M. luteus, P. aeruginosa	158
99	Azadirachta indica (neem)	Polydispersed; less than 40 nm	P. nitroreducens, A. unguis	159
100	Phyllanthus amarus	Flower-liked; 30 nm to 42 nm	E. coli, P. spp., B. spp., S. spp., A. niger, A. flavus, P. spp.	160

several microbes. Verma *et al.*<sup>66</sup> reported *Azadirachta indica* (neem) leaf inspired synthesis of AgNPs and evaluated the effects of pH of the solution on the formation of nanoparticles as change in pH affects the shape and size of the particles by altering the charge of biomolecules, which might affect their capping as well as stabilizing abilities. They have observed that as the pH increases from 9 to 13, the absorption maximum shifts from 383 to 415 nm in the UV-spectrum and detects an

increase in absorption intensity with increasing pH. This showed that pH 13 is the most favourable pH for the synthesis of AgNPs leaf extract. The shift in the peak wavelength indicates that the size of the particles increases with increasing pH of the solution. As the particles' diameter gets larger, the energy required for excitation of surface plasmon electrons decreases, as a result the absorption maximum shifted towards the longer wavelength region. Moreover, it was observed that at acidic pH

S. Aureus

B. Subtilis

B. Subtilis

B. Subtilis

Control

Streptomycin

Gentamicin

Fig. 3 Bactericidal activity of *Prosopis farcta* extract mediated Ag-NPs against human pathogens. This figure has been reproduced from ref. <sup>51</sup> with permission from Elsevier, copyright 2015.

i.e. pH < 7, the formation of nanoparticles is suppressed. At high pH, the bioavailability of functional groups in *Azadirachta indica* leaf extract promoted the synthesis of nanoparticles. However, at very high pH i.e. pH  $\sim$ 13, the particles became unstable and agglomerated, when kept for overnight.

AgNPs were also recently synthesized using several leaf extract of plants such as Artocarpus altilis,67 Crotalaria retusa,68 Cardiospermum halicacabum, 69 Psidium guajava, 70 Cassia fistula 71 and Terminalia chebula.72 In 2016, Anandalakshmi et al.73 reported Pedalium murex leaf extract mediated AgNPs. The produced NPs were tested against several microbes and displayed highest ZOI of 10.5 mm (in 15  $\mu$ L mL<sup>-1</sup> scale) against E. coli and P. aeruginosa and least activity against Klebsiella pneumoniae (8.5 mm). The shape and size of the resultant AgNPs were elucidated with the help of TEM. The TEM micrographs showed that the sizes of the particles were around 50 nm and were predominantly spherical in shape. The PXRD pattern showed fcc crystal structure. Azadirachta indica promoted synthesis of AgNPs was reported by Ahmed et al.74 The produced NPs displayed equal efficacy (9 mm ZOI) against E. coli, S. aureus whereas the plant extract show no antimicrobial activity.

Croton bonplandianum mediated AgNPs were also found to be highy active against microbes.<sup>75</sup> The minimum inhibitory concentrations of synthesized AgNPs were found to be 50, 45, 75 g mL<sup>-1</sup> in case of *E. coli*, *P. aeruginosa*, and *S. aureus* respectively. It was concluded that Gram-negative strains of bacteria with thin cell wall such as *E. coli* and *P. aeruginosa* are more susceptible to cell wall damage compared to Grampositive strain bacteria with a thick cell wall (*S. aureus*). In another work, *Tamarix gallica* leaf extract was used for synthesis of AgNPs. To test its activity against *E. coli*, three sterile filter paper discs (5 mm diameter) were impregnated with 6 μL of AgNPs produced with 5 mL of *Tamarix gallica* extract and 10 mL of 5 mM AgNO<sub>3</sub> solution, López-Miranda *et al.* studied the green synthesis of AgNPs using and evaluated the effect of extract and AgNO<sub>3</sub> concentration on the synthesis.<sup>76</sup> They have observed an

increase in the intensity of surface plasmon resonance (SPR) with the increase in extract concentration, which is attributed to an increasing number of AgNPs formed. Also, as the AgNO<sub>3</sub> concentration increases, many silver ions are increasingly reduced to AgNPs. However, they have seen that the SPR band intensities are nearly independent for 5, 7, and 9 mM AgNO<sub>3</sub>, which reflected that the reaction is close to an equilibrium system because the reducing compounds and stabilizers from the extract are completely consumed, hence it is impossible to reduce a larger amount of silver ions. Henceforth, from the UVvis analysis they concluded that the best results were obtained for the sample 0.15 g mL<sup>-1</sup> extract with 5 mM AgNO<sub>3</sub>. The produced showed 9 mm ZOI against E. coli. Similarly, leaf extract of Urtica dioica,77 Ziziphus oenoplia78 and Lawsonia inermis<sup>79</sup> are reported for the production of AgNPs with high antimicrobial activities. In 2016, a remarkable work on the synthesis of AgNPs using Urtica dioica leaf extract that showed excellent synergistic effect with known antimicrobial drugs was reported by Jyoti et al.77 Interestingly, the synthesized AgNPs apart from showing high antimicrobial activities against several microbes, showed excellent synergistic effect in combination with antibiotics and displayed higher antibacterial effect as compared with AgNPs alone. A high 17.8 fold increase in ZOI was observed for amoxicillin with AgNPs against S. marcescens proving the synergistic role of AgNPs.77 This work provides helpful insight into the development of new antibacterial agents to fight against several new stain of microbes resistant to existing antibiotic drugs. Fig. 4 displayed the synergistic effect of AgNPs and common antimicrobial drugs. The synergistic interaction between AgNPs and antibiotic drugs has been clearly identified using UV-Vis and Raman spectrometer by McShan et al.80 The authors claimed that this synergistic interaction speed up the ejection of Ag+ from AgNPs which inturn boost its antimicrobial activities.

Recently, Manjamadha et al.81 have reported ultrasonicassisted biosynthesis of spherical AgNPs using Lantana camara L. leaf extract. Biosynthesis of AgNPs using ultrasonication improves the reaction conditions such as reducing reaction time and enhancing the reaction rate. Bactericidal activity of the synthesized AgNPs revealed that it shows excellent antibacterial activity against Gram-positive and Gram-negative bacteria. Leaves of Jatropha curcas collected from Micro model complex, Indian Institute of Technology Delhi campus was used for the production of AgNPs.82 The transmission electron microscopy (TEM) analysis showed variation in particle shape and size (20-50 nm), whereas the diameter of NPs was found to be in range of 50-100 nm by scanning electron microscopy (SEM). Complete destruction of the microbial cell was visible using TEM examination. The synthesized NPs were tested for their antimicrobial activities and based on ZOI data, the pattern of sensitivity was observed in the order as E. coli > P. aeruginosa > B. cereus > S. enterica = L. monocytogenes > S. aureus.

Salvinia molesta, 83 Sesbania grandiflora, 84 Indoneesiella echioides 85 and Phlomis 86 leaf extract were also useful for the bioreduction of AgNO<sub>3</sub> to AgNPs. An ultra-small AgNPs with an average diameter of 7.39 nm were prepared using Hydrocotyle rotundifolia. 87 The synthesized AgNPs were tested for its

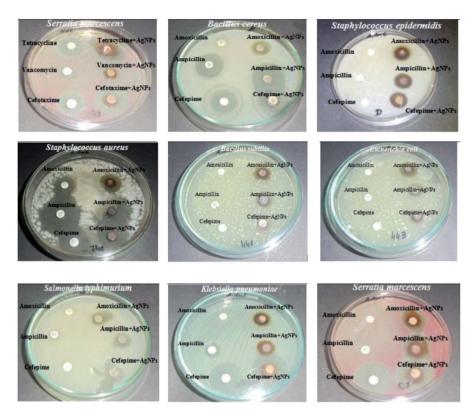


Fig. 4 Synergistic effect of *Urtica dioica* mediated AgNPs with several antibiotics. This figure has been reproduced from ref. <sup>77</sup> with permission from Elsevier, copyright 2016.

antimicrobial property against E. coli (DH5a). The MIC value was recorded as 5 µg mL<sup>-1</sup> and demonstrated significant growth inhibition on agar plate. Formation of spherical AgNPs using Maclura pomifera was achieved in 2017 by Azizian-Shermeh et al. 88 The produced NPs (0.1 mg mL<sup>-1</sup> concentration) displayed a very high ZOI of 23.4  $\pm$  0.1 mm against *E. coli*, which is higher than Ampicillin, a well-known antibiotic drug. In the same year, Bhuyan and coworkers at National Institute of Technology Silchar reported Paederia foetida Linn. inspired AgNPs synthesis.89 The order of activities of the AgNPs against tested microbes is B. cereus > E. coli, S. aureus > A. niger. The author claimed that the AgNPs owing to their small size range (5-25 nm) could have easily penetrated the cell membrane, disturbing the metabolism, cause irretrievable damage finally leading to the microbial cell death. Au NPs has also been synthesized but has not shown any antimicrobial activity which testament the higher activity of AgNPs than that of Au NPs. Biosynthesized AgNPs from leaf extract of Atalantia monophylla,90 Talinum triangulare,91 Ricinus communis,92 Erythrina suberosa, 93 Lippia citriodora, 94 and Brassica oleracea L.95 are also successfully used as an outstanding antimicrobial drug.

In 2017 Al-Shmgani *et al.* prepared AgNPs using *Catharanthus roseus*. <sup>96</sup> They have used identification by color change, UV-vis spectrum, XRD, FTIR, and AFM techniques to confirm the biosynthesis of AgNPs. The leaf extract color changes from yellowish to reddish-brown after adding 2 mM AgNO $_3$  and exposing to heat at 70 °C for 3 min indicating the formation of the NPs. AFM displays the crystalline NPs with grains sized 10–

88 nm in diameter with mean size of about 49 nm. The authors claimed that synthesized AgNPs enter the cell of microbes that resulted in a disruption of adenosine triphosphate (ATP) production and DNA replication, generation of ROS and damage the cell structures as earlier observed by Sahayaraj and Rajesh.<sup>97</sup>

Spherical shape AgNPs with diameter in the range 11–47 nm (by TEM analysis) were produced using Lavandula x intermedia. The AgNPs were found to be most effective against E. coli among all the tested microorganisms shown in Table 1, entry 51. Interestingly, the author also observed that biogenic AgNPs showed ZOI  $23 \pm 0.0$  mm against E. coli whereas streptomycin displayed only  $20 \pm 0.0$  mm under the same concentration. This reflected the high antibacterial efficacy of AgNPs than that of common antimicrobial drug like streptomycin, which could promote its wide use in the future. In another work, a highly crystalline AgNPs were reported to be synthesized from Canna edulis. The NPs showed highest antimicrobial activity against S. typhimurium which is closely related to the finding by Sumitha et al.

In 2017 Artemisia vulgaris mediated AgNPs were reported by Rasheed et al.  $^{100}$  Antimicrobial test revealed that the AgNPs exhibited significant inhibition activities against tested pathogens with the highest value being recorded against S. aureus (18  $\pm$  0.27 mm inhibition zone). Similar to this, earlier in 2016, Thatoi et al.  $^{101}$  reported high activity of AgNPs against S. aureus using AgNPs synthesized from Sonneratia apetala plant extract.

Gram-negative bacteria.

Psidium guajava was applied for the production of spherical AgNPs with average dimension of 25 nm.  $^{102}$  The authors observed that for  $100~\mu g~mL^{-1}$  Psidium guajava mediated AgNPs, the ZOI were  $18.13\pm0.02~mm$  and  $16.92\pm0.18~mm$  against A. faecalis and E. coli, respectively, whereas ZOI of 13.24–14.41~mm were recorded at the same concentration against tested Grampositive bacteria shown in Table 1, entry 54. This finding clearly testament the higher activity of the synthesized AgNPs towards Gram-negative bacteria than the Gram-positive ones. Similar to this finding, earlier in 2013, Geethalakshmi et al. also reported the higher susceptibility of Gram-negative bacteria to silver nanoparticles compared with Gram-positive bacteria.  $^{103}$  Ironically, Psidium guajava mediated AgNPs is however consistently less sensitive towards tested fungi such as S. cerevisiae, A. niger and R. oryzae as compared to both Gram-positive and

Taraxacum officinale leaf extract mediated AgNPs were proved to exhibit an excellent synergistic antibacterial activity with standard antibiotics (such as oxy-tetracycline, tetracycline, ampicillin, and streptomycin) and showed strong positive response against both *X. axonopodis*, *P. syringae*, a plant pathogens. <sup>104</sup> The combined effect of tetracycline with AgNPs significantly inhibited the growth of selected phytopathogens by increasing ZOI about 40% compared to only antibiotics. The authors are of the opinion that NPs-antibiotic combination and their synergistic action would result in higher penetration in the bacterial cell membrane thereby leads to destruction of various cell organelles and death of bacteria, although the mechanism is not yet fully understood till now.

Lateef *et al.*<sup>105</sup> reported that *Petiveria alliacea* L. mediated AgNPs showed 100% inhibition against *E. coli*, *K. pneumoniae*, *S. aureus*, *A. fumigatus* and *A. flavus*. But only 66.67% inhibition in *A. niger*. In another work, microwave-assisted synthesis of AgNPs using leaf extract of *Nervalia zeylanica* was reported. The authors observed no formation of NPs (monitored using UV-spectroscopy) even after 5 h under RT stirring of the extract and AgNO<sub>3</sub>. However, the nanoparticle formation takes place suddenly after 60 s of microwave irradiation. *Ficus ingens* mediated AgNPs recorded MIC value of 10  $\mu$ g mL<sup>-1</sup> on *E. coli* and 20  $\mu$ g mL<sup>-1</sup> on both *S. typhi* and *B. cereus*<sup>107</sup> which is in close agreement with the earlier report of 10  $\mu$ g mL<sup>-1</sup> for *E. coli*. The AgNPs showed highest inhibition against *E. coli* and least with *S. cereus*. Commercial antibiotic Ciprofloxacin showed better activities than the synthesized NPs.

In general, the reduction in the size of the metallic nanoparticles is expected to increase the antibacterial activity due to significantly large surface area of the smaller nanoparticles. However, the results obtained by Erci *et al.*<sup>108</sup> using *Thymbra spicata* leaf extract is worth discussing. In their study higher antibacterial activity of, say, AgNPs2 (average diameter 70.2 nm) in comparison to AgNPs1 (average diameter 25.1 nm) was recorded. They reasoned that this could be due to the shape of AgNPs2, which have triangles, hexagons, spheres and irregular shapes, whereas AgNPs1 exhibit mostly spherical formation. This interesting finding confirmed the shape-dependent bacterial activity of AgNPs, and support earlier reported protocol.<sup>109</sup> The MIC of 50 μg mL<sup>-1</sup> was recorded for *S. cereus* 

whereas, it was 100 μg mL<sup>-1</sup> for *E. coli*. This finding is in sharp contrast to the work of Kavaz et al. 107 mentioned earlier where Gram-negative bacteria has lower MIC than Gram-positive bacteria. However, Erci et al. 108 defended their finding of the more pronounced effect of AgNPs against Gram-positive bacteria than Gram-negative bacteria based on the structural difference in cell wall composition of Gram-positive and Gramnegative bacteria. Gram-negative cell wall was covered with an outer lipid membrane (lipopolysaccharide), which is more negatively charged than Gram-positive. As is evident from the zeta value, the biogenic silver nanoparticles were also negatively charged and the electrostatic repulsion between the nanoparticles and Gram-negative bacteria hinders particle attachment and penetration into the cell37 However, this postulate is not yet fully understood. Again, as against the finding of Erci et al. 108 the Gram-positive bacteria are less affected by AgNPs (produced from *Indigofera tinctoria*) than Gram-negative bacteria as reported by Vijayan et al.110 The authors credited the presence of large number of peptidoglycan layers on the walls of Gram-positive bacteria than Gram-negative bacteria that have to some extent prevent the nanoparticles entry to cytoplasmic membrane than Gram-negative bacteria. Hence, the true role of chemicals in the cell wall of bacteria needed to be properly investigated to understand the underlying mechanism of the cell death due to NPs.

Another interesting work on the shape-dependent activity of biogenic AgNPs was reported using Trichoderma viride extract where the authors reported a higher antimicrobial activity of penta- and hexagonal NPs than spherical NPs when the size are of similar range.111 The different shape AgNPs such as pentagonal, hexagonal and spherical were synthesized by manipulating physical parameters, temperature, pH, and reaction time. At neutral pH (7), spherical NPs were observed under all reaction conditions. Delightfully, at pH 5.0 and 9.0, rectangular and penta-/hexagonal NPs were obtained at 40 °C after 72 h of incubation. In general, longer is the reaction, bigger is the size of NPs whereas higher temperature always affords a smaller NP. It was also found that triangular shape AgNPs showed better antimicrobial activity compared to that of spherical and rod shaped as it has high percentage of facet (1 1 1) that possess a high atomic density which increases binding efficiency of Ag to sulfur containing components, whereas spherical and rod shaped particles have a high percentage of (1 0 0) facets. 112,113

Recently, *Tecoma stans*, <sup>114</sup> *Salvia leriifolia*, <sup>115</sup> *Leucaena leucocephala* L. <sup>116</sup> and *Selaginella bryopteris* <sup>117</sup> were also reported to produced AgNPs which are mainly spherical in nature. *Galega officinalis* leaf extract mediated AgNPs with size-dependent activities were reported by Manosalva *et al.* <sup>118</sup> AgNPs with 23 nm and 220 nm recorded MIC of 5  $\mu$ L mL <sup>-1</sup> and 30  $\mu$ L mL <sup>-1</sup> respectively against *E. coli* showing the higher activity of the smaller NPs. Interestingly MIC of *S. aureus* (a Gram-positive bacteria) is higher (50  $\mu$ L mL <sup>-1</sup>) than *E. coli* (a Gram-negative bacteria) using 23 nm size AgNPs which implies the higher activity of AgNPs against Gram-negative bacteria.

In the year 2019, antimicrobial fabric tests on the dyed cloths were conducted using AgNPs derived from *Camellia sinensis* (tea leaf) extract where bleached cotton cloths were dyed using the

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NPs colloidal solutions. The attachment of AgNPs on the cloths was confirmed by SEM. SEM images of AgNPs with green tea extract also showed the generation of AgNPs. The AgNPs showed excellent antimicrobial activities against S. aureus, K. pneumoniae in the cotton fabric which potentially endorse the suitability of using AgNPs as an effective antimicrobial in cloths.119 Bernardo-Mazariegos et al. used DLS to measure the average hydrodynamic size and zeta potential of the AgNPs synthesized from Justicia spicigera. 120 The sample with a mixture of AgNPs of different sizes gave two broad peaks and was weighted toward the larger particles (z-average size of 4.04 μm and 192 nm). The authors are of the opinion that DLS measurement may not be accurate for polydisperse samples due to its nature to respond toward larger particles. Additionally, the zeta potential was of the NPs was found to be 0.2 mV that indicated the less stability and hence, a tendency to agglomerate to form large particles.

In recent times, highly antimicrobial AgNPs were synthesized using Kleinia grandiflora, 121 Eucalyptus citriodora, 122 Juniperus procera<sup>123</sup> and Capparis zeylanica.<sup>124</sup> Two different shapes structure in the form of sphere and cubic are observed in SEM analysis of the AgNPs generated from Juniperus procera leaf extract. The produced NPs recorded the highest ZOI against P. mirabilis measured at 29  $\pm$  1.3 mm. The author suggested that the high antimicrobial activity of the NPs is due to the inherent activity of the NPs coupled with the plant particulates attached to the NPs, as the plant which contain high flavonoids and polyphenols are a well-known antimicrobial by themselves. 125 Small size AgNPs (9 nm) synthesized using Caesalpinia pulcherrima leaf extract were found to exhibited an MIC as low as  $0.078 \text{ mg mL}^{-1}$  and  $0.156 \text{ mg mL}^{-1}$  for K. pneumoniae and E. coli respectively. Accordingly, the AgNPs possessed maximum antimicrobial activity against K. pneumoniae and E. coli whereas only moderate effects were shown against C. xerosis, S. mutans, S. aureus, S. viridians, S. pyrogenes, S. viridians and C. diphtheriae that have higher MICs.126

Synergistic antimicrobial activity of *Ligustrum lucidum* mediated AgNPs and Epoxiconazole under different conjugation ratio was studied against *S. turcica*, a common maize pathogen.<sup>127</sup> The antifungal activity of AgNPs was evaluated alone, and the synergistic inhibition effect was also measured at various conjugation ratios of AgNPs and epoxiconazole, where a prominent synergistic antifungal effect was observed at 8:2 and 9:1 (AgNPs/epoxiconazole) and the inhibition toxicity ratio reached as high as 1.22 and 1.24, respectively.

Aesculus hippocastanum (horse chestnut) mediated special AgNPs with size  $50\pm 5$  nm was reported to have highest antimicrobial activity (ZOI  $20.0\pm 0.00$  mm) against a Gram-negative bacteria *P. aeruginosa* among all the tested microorganisms listed in Table 1, entry 74. Interestingly, although the AgNPs have profound effects on all the tested bacteria, it have no effect against fungal strains such as *C. albicans* ATCC 10231, *C. tropicalis* ATCC 13803 and *C. krusei* ATCC 1424. The MIC and MBC of AgNPs for the tested microorganisms were in the range from 0.19–12.5 μg mL $^{-1}$  and 1.56–25 μg mL $^{-1}$ .

Ramadan et al.<sup>129</sup> studied the antiviral activity of green synthesized AgNPs and found that AgNPs greatly enhanced the

antiviral activity of *M. alternifolia* leaf extract, which on its own has no effect on the tested viruses such as herpes simplex virus type 1 (HSV-1), and herpes simplex virus type 2 (HSV-2). In addition, the NPs showed excellent activities against several persistent skin bacteria including *S. epidermis* and methicillinresistant *Staphylococcus aureus* (MRSA). Interestingly, tea tree oil of *M. alternifolia* itself showed even higher activity than the AgNPs against some tested microbes which is hardly the case in literature. In another work, *Carya illinoinensis* mediated AgNPs were found to be more efficient against Gram-negative (*E. coli*) than Gram-positive bacteria (*S. aureus*)<sup>130</sup> in a similar trend reported earlier.<sup>118</sup>

Although literature revealed that bacterial cell are generally more sensitive to AgNPs, biogenic AgNPs derived from *Murraya koenigii* leaf extract interestingly shown highly equal activity against Gram-negative bacteria *P. aeruginosa* (ZOI of 18 mm) and a fungus *C. albicans* (18 mm ZOI).<sup>131</sup> In 2020, several leaf extracts of plant such as *Clerodendrum inerme*, <sup>132</sup> *Aspilia pluriseta*, <sup>133</sup> *Melia azedarach*, <sup>134</sup> *Scoparia dulcis*, <sup>135</sup> and *Lantana trifolia*. <sup>136</sup> All these AgNPs are shown to exhibit an excellent antimicrobial activity against numerous common pathogenic microbes. *Mikania micrantha* leaf extract mediated AgNPs were also reported to show a high ZOI of 26.17 mm and 26.05 mm against *B. subtilis* and *E. coli* respectively. <sup>137</sup>

More recently, AgNPs of average size 3.46 nm were produced using Solanum nigrum plant leaf extract. 138 This is one of the smallest biogenic AgNPs reported so far and NPs as small as 1.74 nm were observed. SPR bands band at 442 nm in UV-visible spectroscopy confirmed the formation of AgNPs. Interestingly, the authors observed a much prominent antimicrobial activity exerted by AgNPs compared to AuNPs and PdNPs potentially due to the more effective capping of AgNPs nanoparticles than either Au or PdNPs which results in well-dispersed small AgNPs without much agglomeration as detected by HRTEM. The authors are of the opinion that polyphenols present in Solanum nigrum extract forms a negative environment around the particles and hence create a repulsive force which overcomes the van der Waals force of attraction and prevent AgNPs agglomeration. The AgNPs showed 22 mm ZOI, while 20 mm and 19 mm ZOI are observed in Au and Pd NPs respectively against E. coli at 10 μL mL<sup>-1</sup> concentration. However, although the authors credit the effective capping of AgNPs as a reason for its higher antimicrobial activity, it may also be due to the smaller size of the AgNPs (3.46 nm) as compared to Au (9.39 nm) and Pd NPs (21.55

Maghimaa et al. 139 reported biosynthesis of AgNPs using Curcuma longa leaf extract and investigate their antimicrobial activity in AgNP coated cotton fabric. The loading of AgNPs on the cotton fabric was confirmed by SEM analysis, which was further assisted by the EDX analysis. The authors have reported that the cotton fabric loaded with AgNPs showed great resistance to the growth of pathogenic microorganisms and hence they claimed that the cotton fabric loaded with AgNPs synthesized from Curcuma longa can be used for the diverse application in the medical patient as well as in medical workers to resist microbial infection.

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In 2020, green synthesis of spherical AgNPs, CuNPs and FeNPs with size 11–19, 28–35 and 40–52 nm, respectively using *Syzygium cumini* leaf extract was reported. The order of antibacterial property against methicillin- and vancomycinresistance *S. aureus*, *A. flavus* and *A. parasiticus* microbes was found to be Ag- > Cu- > Fe NPs, which linearly relates with the size of the NPs, thereby reinforcing the size-dependent activity of NPs. In addition, the bioproduction of aflatoxins (a family of toxins produced by certain fungi that are found on agricultural crops such as maize (corn), peanuts, cottonseed, and tree nuts) in *A. flavus* and *A. parasiticus* was also significantly inhibited by AgNPs when compared with the Fe and Cu NPs. Interestingly, the pH of the plants extract reduced after the formation of NPs in all the cases. *Cleistanthus collinus* and *Cestrum nocturnum* are also known to have produced AgNPs.

In another work, rice leaf extract was utilized for the biosynthesis of AgNPs with size 16.5 nm.  $^{144}$  Antifungal activity of the synthesized NPs was tested against mycelium and sclerotia of *R. solani*, a fungus that causes sheath blight disease in rice and found that it inhibits the growth of fungus and the growth inhibition is dependent on the concentration of the AgNPs. The MIC values of AgNPs were in the range of 5–10 and 15–20  $\mu g$  mL $^{-1}$  towards fungal mycelium and sclerotia, respectively. Results revealed that growth inhibition at 10  $\mu g$  mL $^{-1}$  AgNPs is 81.7–96.7% for mycelium and 20  $\mu g$  mL $^{-1}$  treatment completely inhibited disease cause by *R. solani*. In a previous investigation, 43.3–73.6% growth inhibition of *R. solani* was observed at a higher concentration of 2 mg mL $^{-1}$  with larger AgNPs with 40–60 nm.  $^{145}$ 

Recently, an ultra sound-assisted AgNPs of size 8 mm were synthesized using *Mentha aquatica* leaf extract as reducing and capping agent. To the best of our knowledge, this is the smallest biogenic AgNPs reported so far. The production of NPs could occur at RT, but ultrasound greatly reduced the reaction time to 10 min whereas RT took 1 h. The authors highlighted that the phenolic compounds in the *Mentha aquatica* leaf extract get oxidized to Quinone in an alkaline condition which provides free electrons for reduction of the Ag $^+$  ion to Ag $^0$  to form the desired AgNPs. Largely due to its ultra-small size, the AgNPs displayed a very low MIC of 2.2  $\mu$ g mL $^{-1}$  for *P. aeruginosa*, which showed its high efficacy against the tested microbe.

Rosemary (*Rosmarinus officinalis* Linn.)<sup>147</sup> and *Ceropegia thwaitesii*<sup>148</sup> leaf extract mediated AgNPs which showed consistent higher activities against Gram-negative bacteria were also reported. Interestingly, *S. flexneri*, *S. typhi*, *B. subtilis*, *M. luteus*, and *P. mirabilis* are more susceptible to AgNPs than *E. coli*<sup>148</sup> which is not very common in literature.

In the year 2015, Gavade *et al.* prepared AgNPs using the leaf extract of *Ziziphus jujuba* under RT.<sup>149</sup> The AgNPs have different shapes with 20–30 nm size as revealed by TEM images. The authors investigated the effect of pH on the size and stability of the NPs, and observed form UV-Visible spectroscopic graphs that absorbance value linearly increases with increasing pH increases from 4 to 9, which indicates the rate of formation of AgNPs increases from acidic to basic medium. In addition, at acidic pH, bands were wider and display red shift which is an indication of increase in particle size. However, in basic

condition, bands were narrow and display blue shift due to decrease in particle size. The rapid formation of AgNPs in neutral and basic pH this may be due to the ionization of the phenolic groups present in the leaf extract.150 The slow rate of formation and aggregation of AgNPs in acidic pH could be related to electrostatic repulsion of anions present in the solution.151,152 Ironically, at basic pH there is a possibility of AgOH precipitation which need to be avoided. 150 Hence, the authors concluded that the optimum condition for the preparation of AgNPs with desired size and stability was neutral medium. The NPs have a zeta potential of -26.4 mV which is an indication of its excellent stability in colloidal state as a zeta potential higher than 30 mV or lesser than -30 mV is indicative of a stable system. 153 The AgNPs showed high efficacy against E. coli and found to be stable for more than 6 months probably due an excellent capping of NPs (indicated by FR-IR) and low zeta potential.

Irregular shape AgNPs of average size 28 nm, 26.5 nm, 65 nm, 22.3 nm and 28.4 nm were prepared from O. tenuiflorum, S. cumini, C. sinensis, S. trilobatum and C. asiatica, respectively.154 Among several tested microbes the highest antimicrobial activity of AgNPs synthesized by S. trilobatum and O. tenuiflorum extracts was found against Gram-positive bacteria S. aureus (30 mm ZOI) and Gram-negative bacteria E. coli (30 mm) respectively. Interestingly, C. sinensis, S. trilobatum and C. asiatica derived AgNPs consistently showed higher susceptibility towards a Gram-positive bacteria S. aureus and Gram-negative bacteria E. coli and K. pneumoniae. These findings clearly shown that some AgNPs are more sensitive towards a Grampositive bacteria whereas some towards a Gram-negative bacteria, hence the question of selective sensitivity of biogenic AgNPs toward Gram-positive or negative bacteria still remains unsolved. Is the selectivity depending on the biomaterial capping agents attached to NPs or the size of NPs? Hence, one may need to consider the biomolecules present in the plant extract or the size of AgNPs to truly understand the selectivity.

A globular shape AgNPs were prepared using *Amaranthus gangeticus* Linn leaf extract in 2015 which exhibited an inhibitory activity towards Gram-positive, Gram-negative bacteria as well as fungus. <sup>155</sup> In another work *Andrographis paniculata* leaf extract produced a rarely reported cubic shape AgNPs. <sup>156</sup> Study on different shape of AgNPs is of great interest due to the shape-dependent activities of AgNPs towards microbes as noted earlier. <sup>109</sup> The AgNPs showed a high ZOI of 21.3  $\pm$  0.4 mm for Gram-negative bacteria *P. aeruginosa* with very low MIC of 3.125  $\mu$ L mL<sup>-1</sup> which testament its high antimicrobial activity. Yao *et al.* <sup>157</sup> noted that the thickness of the peptidoglycan layer of other Gram-negative bacteria such as *E. coli* is somewhat more than *P. aeruginosa*, hence the author, in good agreement with Yao's work, observed a lower ZOI (16.6  $\pm$  0.3 mm) in case of *E. coli*.

Elangovan *et al.*<sup>158</sup> reported the biosynthesis of AgNPs having cubic, pentagonal and hexagonal shape with size range of 68.06-91.28 nm using *Andrographis echioides* leaf extract and investigate its bactericidal activity against several microbes. The result revealed a high ZOI in the case of *E. coli* (28 mm) and *S. aureus* (23 mm) in  $100 \mu \text{g mL}^{-1}$  concentration of AgNPs.

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Azadirachta indica (neem) leaf extract was also reported for the green synthesis of polydisperse AgNPs at RT and evaluated as a potent antimicrobial agent against P. nitroreducens, a biofilmforming bacterium and fungus A. unguis. 159

While most biogenic AgNPs are spherical, a flower-like structure was reported by Ajitha et al. in 2017.160 The AgNPs showed very high activity towards bacterial culture Pseudomonas spp. (ZOI of 11 mm) even at very low AgNPs concentration (8 μL mL<sup>-1</sup>). It is worth note that the AgNPs also consistently displayed a better activity in fungal strain, Penicillium spp. than bacteria such as *E. coli* and *Staphylococcus* spp. which is hardly a case in any literature as bacteria are usually considered more sensitive to AgNPs than fungi.

#### 3.2 From seeds

Plant seed extract also well established for the biosynthesis of nanoparticles. Till date, various seeds extract has been utilized for the biosynthesis AgNPs (Table 2, entries 1-27). Sinapis arvensis seeds mediated AgNPs was reported for more than 83% inhibition of mycelium growth of fungus N. parvum. Inductively coupled plasma spectrometry (ICP) analysis revealed complete reduction of Ag<sup>+</sup> to Ag<sup>0</sup> in more than 95% conversion within 50 days of reaction.<sup>161</sup> In another work, grape seed extract was utilized for the biosynthesis of spherical and polygonal AgNPs with size ranging from 25-35 nm. Bactericidal activity of the synthesized NPs was tested against eight different ocean pathogenic bacteria; however, it showed great inhibition activity only against four bacteria such as V. alginolyticus, V. anguillarum, V. parahaemolyticus and A. punctate. 162 Sumitha et al. 38 reported bio-reduction of AgNO3 to AgNPs using Durio zibethinus seed extract. It is reported that saccharides present in the extract induces the bio-reduction and the amino acids present in the extract stabilized the synthesized AgNPs. Bactericidal activity was tested against different pathogenic bacteria and found that the NPs showed greater activity against S. typhimurium, S. haemolyticus and S. aureus over B. subtilis, E. coli and S. typhi. However, the synthesized NPs showed lesser inhibition compared to the drug Gentamicin against all the mentioned pathogenic bacteria even at a lower dose of Gentamicin.

Pimpinella anisum, 163 Synsepalum dulcificum, 164 Vigna radiate, 165 Dracocephalum moldavica leaf extracts were also successfully applied for the green synthesis of AgNPs. Vigna radiata mediated AgNPs, was found to be more susceptible towards Gram-negative bacteria E. coli (ZOI 20 mm) than Grampositive S. aureus (ZOI 16 mm) due to the higher thickness of the peptidoglycan layer (approx. 80 nm thick) of the cell wall of Gram positive bacteria which is 10 times thicker than the peptidoglycan Gram-negative bacteria, hence is less susceptible to be destroyed by AgNPs. 165

Several reported literatures revealed that the efficiency of AgNPs as antimicrobial agent is extensively dependent on the shape of the nanoparticles. The comparison of spherical, disc like and triangular shaped AgNPs as antimicrobial agent revealed the activity trend follows as spherical AgNPs > disc-like AgNPs > triangular AgNPs. 65,136The highest inhibition effect of 94.1% and 84% were observed at 40 ppm concentration of AgNPs against R. solani and N. parvum respectively, using AgNPs derived from Trifolium resupinatum seeds extract. 167 In a closely related study, Khatami et al. reported more than 86% inhibition of mycelium growth of R. solani at a concentration 25  $\mu$ g mL<sup>-1</sup> (or 25 ppm) of the biogenic AgNPs. 168 Several plant seeds such as Nigella arvensis, 169 Linseed, 170 Embelia ribes, 171 Melissa officinalis172 are applied for the generation of spherical shape AgNPs. While biogenic AgNPs are reported to be more efficient antimicrobial than any other metal NPs in most of the case, it is worth mentioned that the Embelia ribes derived AgNPs is less susceptible to E. coli at showing ZOI of 20 mm against 28 mm ZOI for AuNPs at 250 μL mL<sup>-1</sup> concentration. <sup>171</sup> Although having small size of NPs (6-25 nm), Leucaena leucocephala mediated AgNPs displayed very low toxicity against both E. coli and S. aureus with ZOI of 18 mm and 22 mm (approx.) respectively at 1000 ppm AgNPs concentration. 173 Alpinia katsumadai seeds extract mediated AgNPs showed excellent activities against E. coli and S. aureus than that of P. aeruginosa, 174 whereas those derived from Myristica fragrans are found to be highly sensitive to multidrug-resistant (MDR) Salmonella enterica serovar typhi (S. typhi) where a highest ZOI of 16.4  $\pm$  0.45 was observed at 100 μg μL<sup>-1</sup> concentration of AgNPs. 175

Common skin bacteria such as P. acnes and S. epidermidis are found to be highly inhibited by AgNPs synthesized using Phoenix sylvestris L. The authors also proved that AgNPs is more susceptible to the tested shin bacteria than the seeds extract as well as  $AgNO_3$  solution as can be seen from the ZOI. <sup>176</sup> The high toxicity of Phoenix dactylifera derived AgNPs against Methicillinresistant S. aureus is clearly seen in SEM images (Fig. 5a-d) and HRTEM images (Fig. 5e and f). Cells treated with AgNPs undergo deformities and irregular cell surface (red arrow). Attachment and penetration of NPs and deformities of the outer most layers of cell wall and cytoplasmic membrane are also clearly visible in HRTEM.177

The seeds extracts of plants such as Tectona grandis, 178 Persea americana,179 Salvia hispanica L180 and Trigonella foenum-graecum<sup>181</sup> produced AgNPs with high antimicrobial activities. Interestingly the size of AgNPs depends on the concentration of Persea americana extract where a small NPs was recorded at low concentration of aqueous extract, whereas high concentration results in the formation of larger NPs. 179 Ironically, the AgNPs from Salvia hispanica L showed lower susceptibility towards antibiotic Ampicillin against E. coli and S. aureus although its high ZOI against E. coli (18.5 mm) and S. aureus (14.9 mm) at 7.7 μL mL<sup>-1</sup> concentration. 180

The increase in lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) enzyme concentration were used as a means to visualize the change in physiology and inhibition caused to microbes such as S. aureus (263 U  $L^{-1}$ ) and S. aureus (263 U  $L^{-1}$ ) by Trigonella foenum-graecum mediated AgNPs. 181 This increase in enzyme reflected that bacteria are under stress conditions due to unfavorable environment on treatment with AgNPs. 182 Synergistic behavior of ampicillin with Hibiscus cannabinus seeds produced AgNPs against S. aureus, B. cereus, E. coli was investigated by Adnan et al. in 2020.183 Biogenic AgNPs that possessed high inhibitory effect on biofilms formation in P. aeruginosa, C. violaceum and S. marcescens was reported. 184 The

Table 2 Various seeds, flower, root extract used for the green synthesis of AgNPs and their antimicrobial activity

No.	Plants	Plant parts	Shape and size	Test microorganisms	Ref
1	Sinapis arvensis	Seeds	Spherical; 1–35 nm	N. parvum	161
2	Grape	Seeds	Spherical and polygonal; 25–35 nm	V. alginolyticus, V. anguillarum, V. parahaemolyticus, A. punctata, E. coli, S. dysenteriae, P. Aeruginosa, S. aureus	162
3	Coffea arabica	Seeds	Spherical and ellipsoidal; 20–30 nm	E. coli and S. aureus	41
4	Durio zibethinus	Seeds	Spherical and rod shaped, 20–75 nm	S. typhi, S. typhimurium, E. coli, S. aureus, S. haemolyticus, B. subtilis	38
5	Pimpinella anisum	Seeds	Spherical; 3.2–16 nm	S. pyogenes, A. baumannii, K. pneumoniae, S. typhi, P. aeruginosa	163
6	Synsepalum dulcificum	Seeds	Spherical; 4–26 nm	P. aeruginosa and K. granulomatis, A. flavus, A. fumigatus, A. niger	164
7	Vigna radiata	Seeds	Spherical; 18 nm	Escherichia coli and Staphylococcus aureus	165
8	Dracocephalum moldavica	Seeds	Spherical; 5–50 nm	E. coli, P. aeruginosa, S. aureus, S. marcescens, S. epidermidis, B. subtilis	166
9	Trifolium resupinatum	Seeds	Spherical; 17 nm	R. solani, N. parvum	167
10	Descurainia sophia	Seeds	Spherical; 1–35 nm	A. rhizogenes, A. tumefaciens, R. solani	168
11	Nigella arvensis	Seeds	Spherical; 2–15 nm	S. pyogenes, B. subtilis, S. aureus, E. coli, P. mirabilis, S. typhimurium	169
12	Linseed	Seeds	Spherical; 10–35 nm	S. mutans, S. epidermidis, P. aeruginosa, E. coli, S. aureus, B. subtilis, A. odontolyticus, A. niger	170
13	Embelia ribes	Seeds	Spherical; 5–35 nm	E. coli, S. aureus	171
14	Melissa officinalis	Seeds	Spherical; 34.64 nm	E. coli, B. subtilis, B. vallismortis	172
15	Leucaena leucocephala	Seeds	Spherical; 6–25 nm	P. gigantea, E. taxodii, E. coli, S. aureus	173
16	Alpinia katsumadai	Seeds	Spherical; 12.6 nm	S. aureus, P. aeruginosa, E. coli	174
17	Myristica fragrans	Seeds	Spherical; 25 nm	Multidrug-resistant (MDR) Salmonella enterica serovar typhi (S. typhi)	175
18	Durio zibethinus	Seeds	Spherical; 20–75 nm	S. aureus, S. typhimurium, E. coli, B. subtilis, S. typhi, S. haemolyticus, S. aureus	38
19	Phoenix sylvestris L.	Seeds	Spherical; 40–50 nm	P. acnes, S. epidermidis	176
20	Phoenix dactylifera	Seeds	Spherical; 14–30 nm	Methicillin-resistant S. aureus	177
21	Tectona grandis	Seeds	Spherical; 10–30 nm	B. cereus, S. aureus, E. coli	178
22	Persea americana	Seeds	Spherical; 50 nm	E. coli	179
23	Salvia hispanica	Seeds	Spherical; 1–27 nm	E. coli, S. aureus	180
24	Trigonella foenum-graecum	Seeds	Spherical; 33.93 nm	E. coli, K. pneumoniae, S. aureus, S. typhi, P. aeruginosa, A. flavus, C. albicans, T. rubrum, P. notatum, T. viridiae	181
25	Sesame (Sesamum indicum, L.)	Seeds	Spherical; 6.6–14.80 nm	P. aeruginosa, K. pneumoniae, B. subtilis, S. aureus	214
26	Hibiscus cannabinus	Seeds	Spherical; 7–11 nm	S. aureus, B. cereus, E. coli	183
27	Carum copticum	Seeds	Spherical; 21.48 nm	P. aeruginosa, S. marcescens, C. violaceum	184
28	Coffea arabica	Seeds	Spherical and ellipsoidal; 20–30 nm	E. coli, S. aureus	41
29	Pimpinella anisum	Seeds	Irregular; 16–48 nm	E. coli, S. aureus, A. flavus, C. albicans	185
30	Marigold (Tagetes erecta)	Flower	Spherical; 46.11 nm	S. aureus, B. cereus, S. coli, P. aeruginosa, C. glabrata, C. albicans, C. neoformans	188
31	Nyctanthes arbortristis	Flower	Spherical and oval; 5–20 nm	E. coli	189
32	Caesalpinia pulcherrima	Flower	Spherical; 12 nm	S. aureus, C. glabrata, B. cereus, E. coli, S. typhimurium, C. albicans, C. neoformans B. subtilis, C. rubrum, Pseudomonas aeruginosa, K. pneumoniae	190
33	Alcea rosea	Flower	Spherical; 7.2 nm	E. coli, S. aureus	191
34	Argemone mexicana	Flower	Spherical; 29.34 nm	S. aureus, P. aeruginosa, E. coli, K. aerogenes	192
35	Turnera ulmifolia	Flower	Spherical; 32.42 nm	S. aureus, P. aeruginosa, E. coli, K. aerogenes	192
36	Tecoma stans	Flower	Spherical; 50–60 nm	E. coli, S. aureus	193
37	Moringa oleifera	Flower	Spherical; 8 nm	K. pneumonia, S. aureus	194
38	Syzygium aromaticum	Flower	Polydisperse; 23 nm	Staphylococcus spp. E. coli, Pseudomonas spp., Bacillus spp., A. flavus, A. niger, Penicillium spp.	195
39	Potentilla fulgens	Root	Spherical; 10–15 nm	E. coli, B. subtilis	196
40	Alpinia calcarata (ginger)	Root	Spherical; 5–15 nm	P. mirabilis, E. coli, B. cereus, S. aureus	197
41	Erythrina indica Lam	Root	Spherical; 20–118 nm	·	198

Table 2 (Contd.)

No.	Plants	Plant parts	Shape and size	Test microorganisms	Ref.
				S. aureus, M. luteus, Escherichia coli, B. subtilis, S. typhi, S. paratyphi	
42	Diospyros paniculata	Root	Spherical; 14–28 nm	P. notatum, A. flavus, A. niger, C. albicans, S. cerevisiae	199
43	Diospyros sylvatica	Root	Spherical; 8 nm	B. pumilis, P. aeruginosa, B. subtilis, S. aureus, K. pneumoniae, E. coli, S. pyogenes, P. vulgaris, A. niger, P. notatum, A. flavus, S. cerevisiae, C. albicans	200
44	Annona muricata	Root	Spherical; 15.08-33.11 nm	K. pneumonia, S. aureus	201
45	Cibotium barometz	Root	Spherical; 23 nm	Escherichia, S. aureus, S. enterica, P. aeruginosa	202
46	Diospyros assimilis	Root	Spherical; 14–28 nm	B. pumilis, B. subtilis, S. aureus, S. pyogenes, K. pneumoniae, E. coli, P. aeruginosa, P. vulgaris, A. niger, A. flavus, C. albicans, P. notatum, S. cerevisiae	203
47	Pelargonium endlicherianum Fenzl.	Root	Spherical; 25–80 nm	P. aeruginosa, E. coli, S. epidermidis	204
48	Rheum palmatum	Root	Spherical; 121 $\pm$ 2 nm	S. aureus, P. aeruginosa	205
49	Lepidium draba	Root	Spherical; 20–80 nm	S. aureus, B. cereus, S. typhimurium, E. coli	207
50	Angelica pubescens Maxim	Root	Quasi-spherical; 12.48 nm	E. coli, S. aureus, P. aeruginosa, and S. enterica	208
51	Phoenix dactylifera	Root	Spherical; 15-40 nm	E. coli, C. albicans	209
53	Arctium lappa	Root	Spherical; 21.3 nm	E. coli, A. tumefaciens, L. acidophilus, S. aureus	210
53	Asparagus racemosus	Root	Spherical; 10–17 nm	E. coli, S. aureus, B. subtilis, K. pneumonia, P. fluorescence, A. hydrophila, E. tarda, F. branchiophilum, Y. ruckeri	211
54	Lysiloma acapulcensis	Root	Spherical; 1.2 to 62 nm	E. coli, P. aeruginosa, S. aureus, C. albicans	212
55	Raphanus sativus	Root	Irregular; 3.2–6.0 nm	S. aureus, E. coli, C. albicans, C. glabrata, C. tropicalis	213

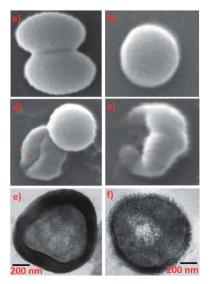


Fig. 5 (a and b) Untreated control cells, (c and d) cells treated with 25 and 50  $\mu g~mL^{-1}$  of AgNPs respectively, SEM images; (e) untreated control cell and (f) treated with 50  $\mu g~mL^{-1}$  AgNPs TEM images. This figure has been adapted from ref.  $^{177}$  with permission from Hindawi, copyright 2018.

biofilms of *P. aeruginosa* was inhibited by 10.6, 18.8, 36.1, 62.0, and 77.6% in presence of 1, 2, 5, 10, and 15  $\mu g \; mL^{-1}$  of *Carum copticum* mediated AgNPs respectively.

In their effort to synthesize AgNPs from Pimpinella anisum seeds extract, Zayed et al. systematically studied to influence of different parameters such as extraction solvent used, extraction temperature, solvent/plant ratio and extraction time which are crucial for the successful synthesis of AgNPs. 185 Hexane, methylene chloride, 70% methanol and water were evaluated as an extraction solvent and 70% methanol was chosen as a best solvent for the fast synthesis of NPs indicated by color change of the reaction solution, whereas this color change is very slow or not visible in other solvent extracts. The high reactivity of 70% aqueous methanol extract towards the reduction of Ag<sup>+</sup> to Ag<sup>0</sup> NPs is due to an excellent solubility of polyphenols in the plant seeds which is efficiently washed down during the extraction process. The SPR peak intensities of both AgNPs and AuNPs increased as the extraction temperature is raised from 25 to 60 °C. This may be due to increasing the solvent's diffusion rate into plant tissues by destroying the cell structures with raising the temperature.31 They also observed increasing SPR by increasing the extraction temperature 25-60 °C but extraction at 60 to 85 °C resulted in decreasing SPR probably due to decomposition of bioreductant at high temperature. The solvent/plant ratio of 10 mL g<sup>-1</sup> was optimized for the AgNPs

synthesis. Increasing the ratio from  $3{\text -}10~\text{mL g}^{-1}$  inceased the SPR due to increasing solubility of biomolecule, however above 10, SPR went down due to high dilution of the extract. The band intensity reached its maximum value with extracts prepared at 60 min, further increase in contact time caused a decrease in the band intensity. It was observed that as extraction time increases the mass transfer coefficient between the solute and solvent increases that potentially increase the amount of the extracted biomolecule from plants which enhance the formation of the NPs. <sup>186</sup> However, prolonged extraction time resulted in the thermal decomposition and oxidation of reactive biomolecules due to prolong heating. <sup>187</sup>

Similarly, the dried and roasted coffee (*Coffea arabica*) seed was employed as a reducing and stabilizing agent for the biosynthesis of AgNPs. TEM micrographs of synthesized AgNPs (Fig. 6) were revealed that the nanoparticles are spherical and ellipsoidal in structure with size ranging from (a) 10–40 nm for 0.1 M, (b) 10–50 nm for 0.05 M and (c) 20–150 nm for 0.02 M. The biomolecules that act as a capping agent around the NPs are visible in TEM images. The SAED patterns indicated that the

nanoparticles are crystalline in nature with a certain d-spacing corresponds to fcc structure. The authors investigated its bactericidal activity against *E. coli* and *S. aureus*. The results revealed that AgNPs solution of 0.05 M and 0.1 M showed a high ZOI in both cases. However, ZOI is higher against *E. coli*.<sup>41</sup>

#### 3.3 From flowers

Recently, flower extracts have been immensely utilized for the biosynthesis of NPs. There are various literatures available for the bio-reduction of AgNO<sub>3</sub> to AgNPs (Table 2, entries 28–37). Padalia *et al.*<sup>188</sup> reported the utilization of flower extract of *Tagetes erecta* for the bio-reduction of AgNO<sub>3</sub> to synthesized AgNPs and investigate their bactericidal activity against both Gram-positive and Gram-negative bacteria such as *S. aureus*, *B. cereus*, *S. coli*, *P. aeruginosa*, *C. glabrata*, *C. albicans*, *C. neoformans*. The result obtained revealed that the bactericidal activity is greater for *E. coli* and *P. aeruginosa* compared to other pathogenic bacteria. Apart from that, the authors reported that the antifungal activity of the AgNPs along with antibiotic against the fungal strain and Gram-

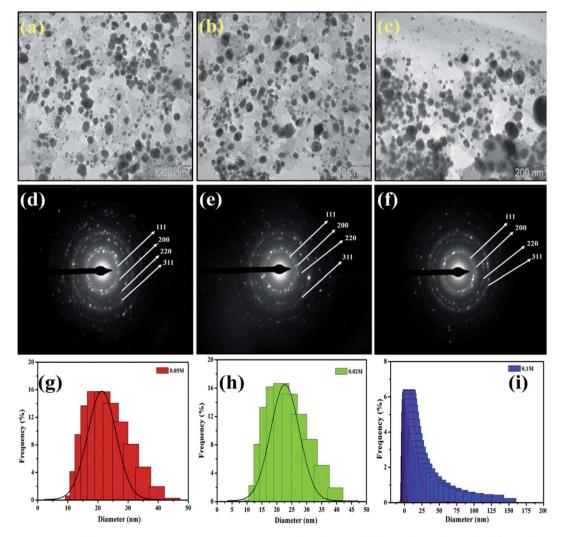


Fig. 6 TEM micrographs and SAED pattern of AgNPs with concentration 0.1 M (a and d), 0.05 M (b and e) and 0.02 (c and f) respectively. This figure has been reproduced from ref. <sup>41</sup> with permission from Elsevier, copyright 2016.

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negative bacteria showed great activity compared to antibiotic alone. Flower extract of plants such as Nyctanthes arbortristis, 189 Caesalpinia pulcherrima, 190 Alcea rosea 191 and Argemone mexicana<sup>192</sup> were also reported from the synthesis of biogenic AgNPs. Tecoma stans flower extract was employed for the biosynthesis of spherical AgNPs with size ranging from 50-60 nm. Antimicrobial activity was tested against S. aureus and E. coli and found that ZOI is higher for S. aureus (24 mm) over E. coli (16 mm). 193 Moringa oleifera generated ultra-small AgNPs that showed a high ZOI of 29 mm against S. aureus. 194 This is one of the highest ZOI observed at this concentration so far using biogenic AgNPs, probably due to its small size. Another exciting finding in this work is the higher antimicrobial activity of the AgNPs in Grampositive bacteria (S. aureus) than the Gram-negative one (K. pneumonia), which is a rare case. In another work, Ajitha et al. reported the biosynthesis of polydisperse AgNPs using Syzygium aromaticum flower extract as bio-reducing as well as a capping agent. Antimicrobial activity of the synthesized NPs was explored against several microbes and found that the NPs induces cell disruption of the bacterial strain and it is maximum in case of Pseudomonas spp. 195

#### 3.4 From roots

Green synthesis of AgNPs and their application as antimicrobial using plants root extract have gained immense attention nowadays (Table 4, entries 38-54). The root extract of Potentilla fulgens was reported as a potent antimicrobial agents against E. coli, B. subtilis showing a ZOI of 9.5  $\pm$  0.2 and 9.7  $\pm$  0.6 respectively.196 Recently, Alpinia calcarata root extract was utilized as a bio-reducing as well as a stabilizing agent for the green synthesis of spherical AgNPs. Antimicrobial activity was tested against P. mirabilis, E. coli, B. cereus, S. aureus and the results showed that Alpinia calcarata root extract assisted synthesized AgNPs have great potential to induce cell disruption of the bacterial strain. Apart from that, the synthesized AgNPs is stable for up to six months.197 AgNPs with microbial activities were also reported to be produced from the root extract of Erythrina indica L. 198 Diospyros paniculate 199 and Diospyros sylvatica.200

Ezealisiji *et al.* have reported the green synthesis of AgNPs using root bark extract of *Annona muricata* Linn and investigate their application as an antimicrobial agent against pathogenic bacteria such as *B. subtilis, S. aureus*, and *K. pneumonia, E. coli*, and *Pseudomonas aeruginosa*. The zone of inhibition (ZOI) in diameters were 10.00, 15.00 mm and 12.50, 17.50, 20.00 mm for the five pathogens respectively at AgNPs concentration of 5  $\mu$ g mL<sup>-1</sup>. The ZOI is increased to 12.50, 14.50 mm and 14.00, 18.50, and 26.00 mm respectively at AgNPs dose of 10  $\mu$ g mL<sup>-1</sup>. Taking into account, the authors have reported that the bactericidal activity of AgNPs is concentration-dependent.<sup>201</sup>

Cibotium barometz,<sup>202</sup> Diospyros assimilis,<sup>203</sup> Pelargonium endlicherianum Fenzl.<sup>204</sup> roots derived AgNPs were also highly sensitive towards tested microorganisms. Diospyros assimilis derived AgNPs showed high ZOI (18 mm approx. at 100 µL mL<sup>-1</sup> AgNPs concentration) against *E. coli* and *S. aureus*; however, they showed lower activity than antibiotic chloramphenicol.<sup>203</sup>

Interestingly the AgNPs derived from *Pelargonium endlicherianum* Fenzl. seed extract (using 11% ethanol extract contain gallic acid and apocynin as major phytochemicals) are monodisperse, whereas those prepared from 70% methanol extract containing gallic acid, apocynin, and quercetin as major components afforded polydisperse NPs as shown in Fig. 7. These indicated the effect of extract solvent on the composition of the extract and nature of the synthesized AgNPs, which have further bearing on the antimicrobial activities of the NPs.<sup>204</sup>

Protein leakage and SEM studies were used as means to study the bactericidal activities of the AgNPs using Rheum palmatum seeds extract.205 SEM images showed abnormality in the cell wall of the tested bacteria, whereas protein was found to leak in high amount due to disruption of membrane in the bacteria when treated with AgNPs which showed the damage caused by AgNPs, which is supported by previous literature.206 The AgNPs showed higher susceptibility towards P. aeruginosa  $(14.35 \pm 0.24 \text{ mm ZOI})$  than S. aureus  $(10.12 \pm 1.81 \text{ mm})$ . The antimicrobial activities of AgNPs from seeds extract of Lepidium draba,207 Angelica pubescens Maxim,208 and Phoenix dactylifera209 were also proven. Interestingly, Angelica pubescens mediated AgNPs showed excellent activities whereas AuNPs and root extract do not possess antimicrobial activity against the tested Gram-negative and Gram-positive bacterial strain.208 Green synthesized AgNPs and AuNPs using Arctium lappa as potent antimicrobial agents are of great interest considering the shape and size of the NPs produced. While AgNPs are mainly spherical with average size 21.3 nm, AuNPS are with different shapes such as spherical, hexagonal and triangular geometry with average size of 24.7 nm were seen in TEM. The authors believed these differences in shape and size of AgNPs and AuNPs are due to the difference in reduction potential as well as the capping agents specific to each NPs.210 In another work, the ZOI of Asparagus racemosus mediated AgNPs were 17.0  $\pm$  0.89 and 16.0  $\pm$  0 for S. aureus, B. subtilis respectively. However, the AgNPs showed low ZOI (12.33  $\pm$  0.51 mm) *E. coli*.<sup>211</sup>

Lysiloma acapulcensis extract was utilized for the green synthesis of AgNPs with size ranging from 3.2–6.0 nm.<sup>212</sup> It is reported that Lysiloma acapulcensis plant is widely used as a traditional medicine in Mexico for the treatment of microbial contamination. Thus, the authors reported that Lysiloma

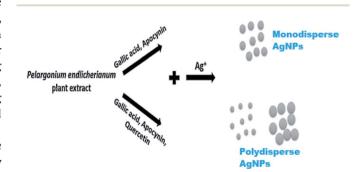


Fig. 7 AgNPs formation when Ag + ions were separately mixed with (gallic acid + apocynin) and (gallic acid + apocynin + quercetin). This figure has been adapted from ref. <sup>204</sup> with permission from Elsevier, copyright 2017.

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acapulcensis root extract mediated AgNPs have higher antimicrobial activity. Antimicrobial activity was tested against the different microorganisms such as E. coli, P. aeruginosa, S. aureus, C. albicans and found that the inhibition potency is in the order E. coli  $\geq$  S. aureus  $\geq$  P. aeruginosa > C. albicans.

Irregular, triangular nanoplates with nanorods, and spherical with average size 6-20 nm, 50-450 nm, 5-30 nm respectively recorded for seed extract, starch, and CTAB-capped AgNPs from Raphanus sativus, which reflected the crucial influence of capping agents on the size and shape of final NPs. In this study, the average NPs size were measured by dynamic light scattering (DLS) technique. The magnitude of the change in the hydrodynamic radius of CTAB-capped AgNPs lower than both extract and starch-capped ones in DLS measurement; hence, the authors proved CTAB is the best shape-directing agent.<sup>213</sup>

#### 3.5 From fruit

The plant fruit extract is widely investigated in the field of green synthesis of nanoparticles.214 There are numerous literature available on the green synthesis of AgNPs employing fruit extracts (Table 3, entries 1-36). Emblica officinalis, 215 guava, 216 carambola,217 Helicteres isora218 and Solanum trilobatum219 fruit extract were utilized successfully for the bio-genic synthesis of spherical AgNPs to investigate its various microbial pathogens such as S. aureus, B. subtilis, E. coli, K. pneumonia, S. mutans, B. cereus and S. typhi. It is reported that, with increase in the concentration of the fruit extract, size of the AgNPs decreases and subsequently antibacterial activity increases.218 Emblica officinalis showed excellent antibacterial activity against Gramnegative bacteria compared to Gram-positive bacteria.215 Due to the very small size of Emblica officinals, guava and Helicteres isora mediated AgNPs, they showed great ZOI against the mentioned pathogens.215,216,218 Apart from that, Lemon,220 Syzygium alternifolium Walp<sup>221</sup> and Nothapodytes nimmoniana<sup>222</sup> fruit extract were also utilized for the green synthesis of AgNPs. Shape directive CTAB was utilized with lemon extract to control the shape of the AgNPs, which was utilized directly to the various bacterial strains and showed excellent activity against the bacterial strains.220 In contrary, both Syzygium alternifolium Walp<sup>221</sup> and Nothapodytes nimmoniana<sup>222</sup> fruit extract act as a shape directive and bio-reducing agent for the biosynthesis of AgNPs. In another work, Lemon extract was utilized as a bioreducing agent for the green synthesis of AgNPs and investigate its antimicrobial activity against four pathogenic bacteria such as P. aeruginosa, E. coli and S. aureus and compared with controls amikacin and lemon extract. The results showed that the synthesized NPs have excellent potency to induce cell disruption of the bacterial strain and ZOI is almost similar to the medicinal antibiotic drug, amikacin.<sup>223</sup> Similarly, spherical AgNPs were also prepared using various fruit extract such as apple extract,224 Adansonia digitata L.225 and Momordica charantia226 extract and examined their bactericidal activity against Gram-positive and Gram-negative bacteria. It is reported that, combined extract-AgNPs exert more bacterial cell damage compared to AgNPs and extract alone due to the synergistic effect produced by the phytochemicals capped on the surface of

the AgNPs.<sup>226</sup> Jayaprakash et al.<sup>227</sup> reported microwave-assisted synthesis of spherical AgNPs using Tamarind fruit extract. The produced AgNPs by this method is very stable without the formation of oxide and displayed excellent bactericidal activity. Moreover, this process for AgNPs synthesis is economic, time efficient and straight forward. Phoenix dactylifera, 228 strawberry,229 Ginseng-berry,230 Kigelia africana231 and Chaenomeles sinensis232 fruit extract were also successfully utilized for the green synthesis of spherical AgNPs with high antimicrobial activity. Ginseng-berry fruit extract derived AgNPs showed greater ZOI against S. aureus (12.3 mm) compared to E. coli (11 mm).230 A high bactericidal activity against the Gram-positive bacteria was observed by using AgNPs derived from Phoenix dactylifera extract.228

Biogenic synthesis of AgNPs are achieved by using various fruit extract such as Soymida febrifuga, 233 Ribes nigrum, 234 Garcinia indica, 235 Carissa caranda berries 236 and Diospyros lotus 237 and investigate their bactericidal activity against various bacterial pathogens. It is reported that biogenic synthesis of AgNPs depends on the various factors such as AgNO<sub>3</sub> concentration, extract to AgNO3 ratio, pH, incubation temperature and time.235 The optimal conditions for green synthesis of AgNPs using Garcinia indica are 1.5 mM AgNO<sub>3</sub>, 1:1 AgNO<sub>3</sub>/Kokum fruit extract, pH 10, incubation temperature of 37 °C and 24 h time.235 Carandas berry mediated AgNPs displayed great ZOI against various Gram-negative bacteria. However, to inhibit the growth of bacteria S. aureus, a comparatively high concentration of AgNPs is required. 236 There are several reports where different fruit extract such as Terminalia bellirica,238 clammy cherry,239 Phyllanthus emblica, 240 Forsythia suspense, 241 Rosa canina 242 and Manilkara zapota (Sapota)243 have been productively utilized for the green synthesis of AgNPs and evaluate their bactericidal activity against various bacterial pathogens. The results revealed that, AgNPs generated from each fruit extract displayed great antimicrobial activity against both Gram-negative and Gram-positive bacteria. It is reported that microwave-assisted synthesis of AgNPs using cherry extract is a time efficient and cost-effective process. The synthesized NPs are very small with size 7. 13 nm, thus displayed easy cell disruption of various human pathogens.239

Besides, antimicrobial activity of various fruit extract such as Abelmoschus esculentus, 244 Phyllanthus emblica, 245 Aegle marmelos, 246 Nauclea latifolia, 247 Myristica fragrans, 248 Capsicum frutescens<sup>249</sup> and Areca catechu<sup>250</sup> mediated AgNPs was also tested and found that the synthesized AgNPs displayed great cell disruption of bacterial strains. The effect of solvent extract of Aegle marmelos on antimicrobial activity was tested by making fruit extract in various solvents such as petroleum, ether, methanol, acetone and chloroform and found that methanol extract of Aegle marmelos displayed highest cell disruption against B. cereus and lowest for E. coli.246

#### 3.6 From gum

Gum extracts of various plants have been widely utilized as a bio-reducing as well as a stabilizing agent for the green synthesis of AgNPs (Table 3, entries 37-45). Velusamy et al.251

Table 3 Various fruit extract used for the green synthesis of AgNPs and their antimicrobial activity

No.	Plants	Plant part	Shape and size	Test microorganisms	Ref
1	Emblica officinalis	Fruit	Spherical; 15 nm	S. aureus, B. subtilis, E. coli, K. pneumonia	215
2	Psidium guajava	Fruit	Spherical; 2–10 nm	S. mutans, B. cereus, E. coli, S. aureus, and S. typhi	216
3	Carambola	Fruit	Spherical; 10–40 nm	E. coli, P. aeruginos	217
4	Helicteres isora	Fruit	Spherical; 8–20 nm	P. aeruginosa	218
5	Solanum trilobatum	Fruit	Spherical; 12.50–41.90 nm	S. mutans, E. faecalis, E coli, K. pneumoniae	219
6	Lemon	Fruit	Spherical and polyhedral; 15–30 nm	S. aureus, E. coli, Candida albicans, Candida glabrata and Candida tropicalis	220
7	Syzygium alternifolium	Fruit	Spherical; 5–68 nm	B. subtilis, S. aureus, E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. typhimurium, A. solani, A. flavus, A. niger, P. chrysogenum, T. harzianum	221
8	Nothapodytes nimmoniana	Fruit	Spherical; 44–64 nm	B. subtilis, E. coli, S. aureus, S. paratyphi, P. vulgaris, A. hydrophillus, K. pneumoniae	222
9	Citrus lemon	Fruit	Spherical; 2–10 nm	P. aeruginosa, E. coli, S. aureus	223
10	Apple	Fruit	Spherical; $30.25 \pm 5.26$ nm	E. coli, S. aureus	224
11	Adansonia digitata L.	Fruit	Spherical; 3–57 nm	P. vulgaris, K. pneumoniae, P. aeruginosa, S. typhimurium, E. coli, B. subtilis, S. aureus. T. harzianum, A. niger, A. flavus, P. chrysogenum, A. solani	225
12	Momordica charantia	Fruit	Spherical; 78.5–220 nm	E. coli, S. typhi, S. aureus, P. aeruginosa	226
13	Tamarindus indica (Tamarind)	Fruit	Spherical; 6–8 nm	B. cereus, S. aureus, M. luteus, B. subtilis, Enterococcus sp., P. aeruginosa, S. typhi, E. coli, K. pneumonia	227
14	Phoenix dactylifera	Fruit	Spherical; 25–60 nm	E. coli, K. pneumonia, S. epidermidis, (d) B. cereus, S. aureus	228
15	Strawberry	Fruit	Spherical; 7–65 nm	P. aeruginosa, B. licheniformis	229
16	P. ginseng Meyer	Fruit	Spherical; 10–20 nm	E. coli, S. aureus	230
17	Kigelia africana	Fruit	Spherical; 10 nm	K. pneumoniae, P. aeruginosa, C. albicans	231
18	Chaenomeles sinensi	Fruit	Spherical; 20 nm	S. aureus, E. coli	232
19	Soymida febrifuga	Fruit	Spherical; 14.27 nm	B. subtilis, E. coli, S. aureus, P. putrida	233
20	Ribes nigrum	Fruit	Spherical; 5–10 nm	S. aureus, P. aeruginosa, E. coli, C. albicans, Trichophyton rubrum, A. niger	234
21	Garcinia indica	Fruit	Spherical, hexagonal; 5–30 nm	E. coli, B. subtilis, S. aureus, P. aeruginosa, Salmonella enterica typhi, P. vulgaris, S. marcescens	235
22	Carissa carandas	Fruit	Spherical; 10–60 nm	A. hydrophila, Acinetobacter sp., S. aureus	236
23	Diospyros lotus	Fruit	Spherical; 19 nm	E. coli, S. aureus	237
24	Terminalia bellirica	Fruit	Spherical; 10 nm	P. aeruginosa, K. pneumoniae	238
25	Cordia obliqua Willd	Fruit	Spherical; 7.13 nm	B. circulans, E. coli, P. aeruginosa, S. aureus	239
26	Phyllanthus emblica	Fruit	Spherical; 19.8–92.8 nm	A. oryzae	240
27	Forsythia suspensa	Fruit	Spherical; $47.3 \pm 2.6 \text{ nm}$	V. parahaemolyticus, S. aureus	241
28	Rosa canina	Fruit	Spherical; 13–21 nm	B. cereus, E. hirae, S. aureus, E. coli, L. pneumophila, Candida albicans, P. aeruginosa	242
29	Manilkara zapota (Sapota)	Fruit	Spherical; 8–16 nm	E. coli, P. aeruginosa, K. pneumoniae, Bacillus subtilis subsp. Spizizenii, S. aureus	243
30	Abelmoschus esculentus	Fruit	Spherical; 3–11 nm	B. subtilis	244
31	Phyllanthus emblica	Fruit	Spherical; 19 nm to 45 nm	K. pneumoniae, S. aureus	245
32	Aegle marmelos	Fruit	Spherical; 10–200 nm	B. cereus, S. aureus, E. coli, P. aeruginosa, S. typhi, S. dysenteriae Y. pestis	246
33	Nauclea latifolia	Fruit	Irregular, 12 nm	E. coli, C. albicans, Rhizopus sp., A. niger, C. fruendii, S. aureus, Staphylococcus sp. Klebsiella sp.	247
34	Myristica fragrans	Fruit	Irregular; 31.31 nm	E. coli, P. aeruginosa, S. aureus, B. subtilis	248
35	Capsicum frutescens	Fruit	Monodispersed; 20–25 nm	E. coli, B. subtilis	249
36	Areca catechu	Fruit	Polydispersed; 12 nm	E. coli, P. aeruginosa, K. aerogenes, S. aureus	250
37	Azadirachta indica L.	Gum	Spherical; 12.09–29.65 nm	S. enteritidis, B. cereus	251
38	Salmalia malabarica	Gum	Spherical; 7 nm	S. aureus and E. coli	252

Table 3 (Contd.)

No.	Plants	Plant part	Shape and size	Test microorganisms	Ref
39	Styrax benzoin	Gum	Spherical; 12–38 nm	P. aeruginosa, S. aureus, E. coli, C. tropicalis	253
40	Anacardium occidentale L.	Gum	Spherical; 51.9 nm	S. aureus, E. coli	254
41	Araucaria heterophylla	Gum	Spherical; less than 50 nm	E. coli, Streptococcus sp	255
42	Azadirachta indica	Gum	Spherical; less than 50 nm	E. coli, Streptococcus sp	255
43	Prosopis chilensis	Gum	Spherical; less than 50 nm	E. coli, Streptococcus sp	255
44	Buchanania lanzan	Gum	Spherical; 14.74–19.86 nm	E. coli, A. avium, S. intermedius, P. macerans, S. rubidaea, E. mallatovora, E. faecalis, S. haemolyticus, P. mirabilis, S. epidermidis S. chromogenes, E. agglomerans, Staphylococcus capitis ssp. capitis, Staphylococcus capitis ssp. urealyticus	256
45	Mimosa pudica	Gum	Irregular; no report	E. coli, S. commune	257
46	Moringa oleifera	Stem	Spherical; 3–70 nm	E. coli, K. cloacae, S. epidermidis	258
47	Waste grass	Stem	Spherical-oblate; 4–34 nm	P. aeruginosa, A. baumannii, F. solani R. solani	259
48	Swertia paniculate	Stem	Spherical; 31-44 nm	P. aeruginosa, K. pneumoniae, S. aureus	260
49	Caesalpinia pulcherrima	Stem	Spherical; 8 nm	B. cereus, S. aureus, C. rubrum, B. subtilis, E. coli, K. pneumonia, P. aeruginosa, S. typhimurium, C. albicans, C. glabrata, C. neoformans	261
50	Garcinia mangostana	Stem	Spherical; 30 nm	K. planticola, E. coli, B. subtilis	262
51	Dorema ammoniacum D.	Stem	Spherical; 28.4 nm	E. coli, S. typhimurium, S. aureus, B. cereus	263
52	Fumariae herba	Stem	Spherical; 25 nm	S. aureus, B. cereus, B. luteus, B. subtilis, L. monocytogenes, E. coli, P. aeruginosa, K. pneumoniae, P. vulgaris, C. albicans	264
53	Anthemis atropatana	Stem	Spherical; 38.89 nm	S. aureus, S. pyogenes, P. aeruginosa, E. coli	265
54	Afzelia quanzensis	Bark	Spherical; 10–80 nm	E. coli, S. aureus	266
55	Syzygium alternifolium	Bark	Spherical; 4–48 nm	B. subtilis, S. aureus, E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. typhimurium, A. solani, A. flavus, A. niger, P. chrysogenum, T. harzianum	267
56	Cochlospermum religiosum	Bark	Spherical; 20–35 nm	Bacillus, E. coli, Proteus, Pseudomonas, Staphylococcus, A. flavus, Fusarium, C. lunata, Rhizopus, A. niger	268
57	Ficus benghalensis	Bark	Spherical; 85.95 nm	E. coli, P. aeruginosa, B. subtilis	269
58	Azadirachta indica	Bark	Spherical; 90.13 nm	E. coli, P. aeruginosa, B. subtilis	269
59	Plumbago zeylanica	Bark	Spherical; 28.47 nm	B. subtilis, P. aeruginosa, S. aureus, C. tropicalis, E. coli, A. flavus	270
60	Helicteres isora	Bark	Spherical; 16–95 nm	E. coli, V. cholera, S. typhi, P. aeruginosa, B. subtilis and M. luteus	271
61	Terminalia arjuna	Bark	Spherical; 65 nm	E. coli	272
62	Butea monosperma	Bark	Spherical; 18–50 nm	B. subtilis, E. coli	273
63	Prosopis juliflora	Bark	Spherical; 10–50 nm	E. coli, P. aeruginosa	274
64	Garcinia mangostana	Bark	Spherical; 65 nm	E. coli, B. subtilis, S. aureus, B. cereus, K. pneumoniae	275
65	Solanum trilobatum	Bark	Spherical; 25 nm	A. niger, E. coli, Bacillus sp.	276
66	Butea monosperma (Lam.) Taub.	Bark	Spherical; 81 nm	E. coli, S. aureus, A. niger	277
67	Syzygium cumini	Bark	Spherical; 15 nm	E. coli, B. subtilis	278
68	Diospyros montana	Bark	Spherical; 5–40 nm	K. aerogenes, E. coli, B. subtilis, S. aureus	279
69	Handroanthus impetiginosus	Bark	Spherical; 13.4 nm	S. aureus, E. coli	280

have reported the autoclave-assisted green synthesis of AgNPs using gum extract of *Azadirachta indica* L. AFM analysis of the synthesized AgNPs displayed that the nanoparticles are spherical in shape without any aggregation. Furthermore, the line profile analysis revealed that the average particle size is 23.44 nm. Bactericidal activity test revealed that the synthesized

AgNPs can effectively disrupt the cell membranes of *S. enteritidis* and *B. cereus*, hence can be exploited in biomedical applications. Similarly, the bactericidal activity of AgNPs generated from various gum extract such as *Salmalia malabarica*, 252 Styrax benzoin 253 and Anacardium occidentale L. 254 displayed prominent cell damage against both Gram-positive and

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Gram-negative bacteria. In a comparison study, three different gum extract such as Azadirachta indica, Araucaria heterophylla and Prosopis chilensis have been utilized for the synthesis of AgNPs and compare their antimicrobial activity against both Gram-negative and Gram-positive bacteria. The results revealed that, Azadirachta indica and Prosopis chilensis mediated AgNPs are effective for cell disruption of both Gram-positive and Gramnegative bacterial strains. On contrary, Araucaria heterophylla mediated AgNPs is effective for Gram-negative bacterial strains (E. coli) only.255 In another study, Buchanania lanzan gum extract was utilized for the green synthesis of AgNPs with size ranging from 14.74-19.86 nm. Antimicrobial activity was tested against 14 Gram-negative and 3 Gram-positive bacteria and found that the synthesized AgNPs was more prominent against Gramnegative bacteria over Gram-positive bacteria. Besides, MIC for two Gram-negative bacteria such as E. coli and A. avium was found to be 0.52  $\mu g \ mL^{-1}$  and 0.53  $\mu g \ mL^{-1}$  respectively.<sup>256</sup> Beside, Mimosa pudica gum extract was utilized for the green synthesis of AgNPs and investigate its antimicrobial activity against Escherichia coli and Schizophyllum commune, observed that the ZOI for E. coli is higher than the S. commune. 257

#### 3.7 From stem

Plants stem extract was widely utilized as a reducing agent for the green synthesis of AgNPs (Table 3, entries 46-53). Aqueous extract of Moringa oleifera, 258 waste grass 259 and Swertia paniculate<sup>260</sup> have been utilized for the bio-synthesis of AgNPs to investigate its antimicrobial activity against various bacterial strains. It is reported that the waste grass mediated AgNPs are smaller in size (15 nm) compared to Moringa oleifera and Swertia paniculate mediated AgNPs, hence AgNPs obtained from waste grass extract shows greater antimicrobial activity as it can easily disrupt the bacterial cell wall.<sup>259</sup> Similarly, green synthesis of AgNPs by using C. pulcherrima stem extract was reported by Moteriya et al.261 and examine their antimicrobial activity against various pathogenic microorganisms. The results showed that the MIC value for the bacteria is ranging from 0.312 to 2.5 mg mL<sup>-1</sup> and for fungi is 2.5 mg mL<sup>-1</sup> using AgNPs only. Interestingly, AgNPs together with two antibiotics such as chloramphenicol and amphotericin B recorded a lower MIC value against both bacteria and fungi compared to the bare AgNPs. The combination of AgNPs and chloramphenicol displayed synergistic effect against B. cereus, B. subtilis, S. aureus, C. rubrum and S. typhimurium, while displayed partial synergistic effect against E. coli, P. aeruginosa, K. pneumonia. In addition, green synthesis of AgNPs by using different stem extract of Garcinia mangostana, 262 Dorema ammoniacum D. 263 and Fumariae herba<sup>264</sup> is also reported. The synthesized AgNPs were applied against various bacterial strains of Gram-positive and Gramnegative bacteria and found that the NPs is very active for the cell disruption of Gram-negative bacteria as the Gram-negative bacteria possesses weak cell wall due to the less content of peptidoglycan in the cell wall. In another work, Anthemis atropatana extract was utilized for the biosynthesis of AgNPs and investigated the antimicrobial activity of the produced NPs against various pathogenic bacteria such as S. aureus (ATCC

6538), S. pyogenes (ATCC 19615), P. aeruginosa (ATCC 15442) and E. coli. The result obtained revealed that the highest and lowest MIC value is for P. aeruginosa and S. aureus, respectively.265

#### 3.8 From bark

In recent years, bark extract has been widely exploited as a reducing agent as well as a stabilizing agent for the green synthesis of AgNPs (Table 3, entries 54-69). Green synthesis of AgNPs for antimicrobial activity was obtained by using various plant bark extract Afzelia quanzensis, 266 Syzygium alternifolium 267 and Cochlospermum religiosum.268 Nayak et al.269 have reported the green synthesis of AgNPs with the size of 90.13 nm using bark extract of Ficus benghalensis and Azadirachta indica. Bactericidal activity of the synthesized AgNPs was tested against Gram-positive and Gram-negative bacteria such as E. coli, P. aeruginosa, V. cholera and B. subtilis, observed great inhibition potential of the synthesized AgNPs against the bacterial pathogens. The authors reported that the phytochemicals present around the synthesized AgNPs provide unique surface characteristic and thus, can damage various cell membranes. Apart from that, different plant bark extract such as Plumbago zeylanica,<sup>270</sup> Helicteres isora,<sup>271</sup> Terminalia arjuna,<sup>272</sup> Butea monosperma,<sup>273</sup> Prosopis juliflora,<sup>274</sup> Garcinia mangostana<sup>275</sup> and Solanum trilobatum<sup>276</sup> were also utilized for the green synthesis of AgNPs and investigate its antimicrobial activity against various bacterial strains. It is reported that the Butea monosperma bark extract is inefficient for any ZOI, however, the extract mediated AgNPs displayed great ZOI at very low concentration. The ZOI induced by AgNO3 solution is more compared to AgNPs, but required high concentration of AgNO<sub>3</sub>, which is harmful for the consumer. On contrary, a very small concentration of AgNPs showed good ZOI against the bacterial strains and hence can be used as therapeutic agent.273

Recently, Butea monosperma, 277 Syzygium cumini278 and Diospyros montana<sup>279</sup> bark extract were utilized for the green synthesis of AgNPs and investigate its antimicrobial activity. The resultant AgNPs are displayed prominent cell damage to the various bacterial strains. Syzygium cumini mediated AgNPs displayed greater ZOI against the Gram-negative bacteria compared to Gram-positive bacteria as the cell wall of Gramnegative bacteria is more susceptible for the synthesized AgNPs. The study also revealed that the bactericidal activity of Syzygium cumini mediated AgNPs is more compared to the Syzygium cumini extract and AgNO3 solution, which can attributed to the small size of the AgNPs.<sup>278</sup> To increase the rate of the biosynthesis process for AgNPs, the microwave technique was used by Tormena et al.280 where they have used Handroanthus impetiginosus bark extract as a reducing as well as capping agent. Bactericidal activity of the synthesized NPs was tested against two pathogenic bacteria such as S. aureus and E. coli and found good inhibition potential to both bacterial strains with MIC value 3.1  $\times$  10<sup>2</sup>  $\mu g$  mL<sup>-1</sup> and 6.7  $\times$  10<sup>4</sup>  $\mu g$  mL<sup>-1</sup> respectively. However, the pure extract displayed a low MIC value of 2.7  $\times$  10<sup>3</sup> µg mL<sup>-1</sup> and 1.2  $\times$  10<sup>3</sup> µg mL<sup>-1</sup> for S. aureus and E. coli, respectively. Interestingly, the bactericidal activity of AgNPs is higher for S. aureus compared to E. coli (Fig. 8). This is contrary

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to the generally accepted assumption that AgNPs are more susceptible to Gram-negative bacteria due to their thin cell-wall. However, the authors defended their claim by considering the synergetic effects of biomolecules capping agents and AgNPs.

#### From rhizome

Rhizome extract is also utilized in the green synthesis of sizeselective AgNPs (Table 4, entries 1-8). Bio-synthesis of spherical AgNPs was obtained by using various rhizome extract such as Bergenia ciliate<sup>281</sup> and Dryopteris crassirhizoma.<sup>282</sup> The synthesized AgNPs was utilized against different bacterial strains to investigate its antimicrobial activity and found that the NPs displayed excellent cell disruption of the bacterial strains. A wide variety of LED light source such as green, red and blue light have been utilized for the synthesis of AgNPs and examined its bactericidal activity. The authors reported that the ZOI against B. cereus is maximum when the green light mediated AgNPs is used followed by red and blue light mediated AgNPs.282 Rhizome extract of Coptis chinensis was exploited for the green synthesis of AgNPs with size 15 nm. The surface of the synthesized AgNPs was further modified with chitosan. Bactericidal activity of both free AgNPs and chitosan modified AgNPs was tested against E. coli and B. subtilis. The results revealed that the chitosan modified AgNPs showed greater inhibition efficiency compared to free AgNPs against the bacterial strains.37 Recently, rhizome extract of Coptidis,283 Curcuma longa (turmeric),284 Canna indica L.285 and Ferula foetida (asafoetida gum)286 were utilized for the green synthesis of spherical AgNPs with size in the nanometer range. The resultant AgNPs was tested against various bacterial strains to investigate its bactericidal activity and found that the NPs is effective for the bacterial cell damage. It is observed that the Canna indica L.

mediated AgNPs displayed greater bactericidal activity against E. coli compared to the other bacteria such as S. aureus and K. pneumoniae. However, on comparison of the bactericidal activity of Canna indica L. mediated AgNPs with conventionally antibiotic drug Gentamicin revealed that the NP is less active compared to Gentamicin.285 In another work, Ginger rhizome extract and sodium citrate were utilized for biosynthesis of B-AgNPs and C-AgNPs respectively followed by investigated their antibacterial activity against six aquatic pathogens such as V. anguillarum, V. alginolyticus, A. punctate, V. parahaemolyticus, V. splendidus, V. harveyi. The results revealed that the chemically synthesized AgNPs showed slightly greater ZOI against the six pathogens compared to Oxford cup indicates that both have very weak bactericidal activity. In contrast, ginger rhizome extract mediated AgNPs (B-AgNPs) showed greater ZOI against the 6 aquatic pathogens, which confirmed that B-AgNPs displayed greater bactericidal activity compared to the chemically synthesized AgNPs.287

#### 3.10 From peels

Recently, utilization of peel extract as a reducing agent for the green synthesis of nanoparticles with selective size gaining immense attention. To date, various peel extract was used for the biosynthesis of AgNPs (Table 4, entries 9-17). Peel extract of Cavendish banana, 45 banana (Musa paradisiaca). 288 Carica papaya<sup>289</sup> and Citrus sinensis<sup>290</sup> were utilized for the green synthesis of AgNPs. The synthesized AgNPs were tested against various bacterial strains to investigate its bactericidal activity and found that the AgNPs obtained from various peel extract displayed great cell disruption. The influence of various factors on the bio-reduction of AgNO3 was investigated and found that 1.75 mM AgNO<sub>3</sub>, 20.4 mg dry banana peel 4.5 pH and 72 h

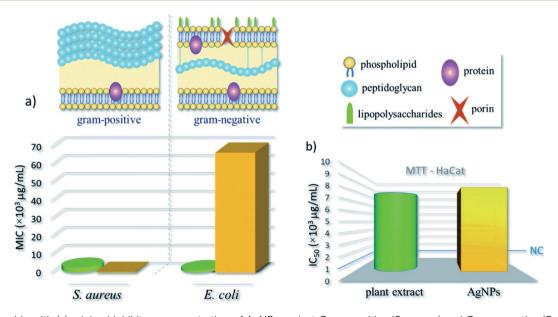


Fig. 8 Infographic with (a) minimal inhibitory concentration of AgNPs against Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria models and (b) half maximal inhibitory concentration (IC<sub>50</sub>) using MTT assay – the blue line indicates the cytotoxicity criteria for preliminary tests of new compounds, as established by the United States' National Cancer Institute (NCI). Green cylinders and yellow bars represent the plant extract and AqNPs results, respectively. This figure has been reproduced from ref. 280 with permission from RSC, copyright 2020

Table 4 Various rhizomes, peel, tube/bulb, whole plant, petals, latex, pod and callus extract used for the green synthesis of AgNPs and their antimicrobial activity

No.	Plants	Plant part	Shape and size	Test microorganisms	Ref
1	Bergenia ciliata	Rhizome	Spherical; 35 nm	M. luteus, S. aureus, E. aerogenes, B. bronchiseptica, A. niger, A. fumigatus, A. flavus, F. solani	281
2	Dryopteris crassirhizoma	Rhizome	Spherical; 5–60 nm	B. cereus and P. aeruginosa	282
3	Coptis chinensis	Rhizome	Spherical; 15 nm	E. coli, B. subtilis	321
4	Coptidis rhizome	Rhizome	Spherical; 30 nm	E. coli, S. aureus	283
5	Curcuma longa (turmeric)	Rhizome	Spherical; $18 \pm 0.5 \text{ nm}$	E. coli, L. monocytogenes	284
6	Canna indica L	Rhizome	Spherical; 20–70 nm	S. aureus, K. pneumoniae, E. coli	285
7	Ferula foetida (asafoetida gum)	Rhizome	Spherical; 5.6–8.6 nm	E. coli, K. pneumoniae, C. albicans	286
8	Zingiber officinale (ginger)	Rhizome	Polygonal; 20–80 nm	V. anguillarum, V. alginolyticus, A. punctate, V. parahaemolyticus, V. splendidus, V. harveyi	287
9	Cavendish banana	Peel	Spherical; 23–30 nm	S. aureus, B. subtilis, K. pneumonia, E. coli	45
10	Banana (Musa paradisiaca)	Peel	Spherical; 23.7 nm	B. subtilis, S. aureus, P. aeruginosa, P. aeruginosa, E. coli, C. albicans	288
11	Carica papaya	Peel	Spherical; 16–20 nm	E. coli, S. aureus	289
12	Citrus sinensis	Peel	Spherical; 48.1 $\pm$ 20.5 nm	X. axonopodis pv. Citri (Xac)	290
13	Citrus maxima	Peel	Spherical; 4–11 nm	E. coli, S. aureus, F. oxysporum, V. dahliae	291
14	Punica granatum	Peel	Spherical; 6–45 nm	E. coli, S. aureus	292
15	Citrus × clementina	Peel	Spherical; 15–20 nm	E. coli, B. cereus, S. aureus	293
16	Solanum melongena	Peel	Spherical; 92.4 nm	P. fluorescens, B. amyloliquefaciens	294
17	Citrus limetta	Peel	Spherical; 18 nm	C. albicans, C. glabrata, C. parapsilosis, C tropicalis, M. luteus, S. mutans, S. epidermidis, S. aureus, E. coli	
18	Allium cepa	Tube/bulb	Spherical; 10 nm	E. coli, P. aeruginosa, B. subtilis, F. oxysporium, F. oxysporum	296
19	Sunroot tuber (Helianthus tuberosus)		Spherical; 10–70 nm	R. solanacearum, X. axonopodis	297
20	Dioscorea alata	Tube/bulb	Spherical; 10–25 nm	E. coli, S. auricularis	298
21	Crocus haussknechtii Bois	Tube/bulb	Spherical;; 10–25 nm	S. aureus, P. aeruginosa	299
22	Allium sativum (garlic)	Tube/bulb	Spherical; 50–70 nm	P. aeruginosa, B. licheniformis	300
23	Sargassum muticum	Whole plant	Spherical; 43–79 nm	B. subtilis, K. pneumoniae, S. typhi	301
24	Brassica oleracea L. (Broccoli)	Whole plant	Spherical; 30–45 nm	E. coli, B. subtilis and S. aureus, Aspergillus sp., Pneumocystis sp.	302
25	Vernonia cinerea L.	•	Spherical; 40–75 nm	C. albicans, Penicillium spp	303
26	Artemisia marschalliana	_	Spherical; 5–50 nm	S. aureus, B. cereus, A. baumannii, P. aeruginosa	304
27	Linum usitatissimum L.		Spherical; 49–54 nm, 19–24 nm respectively	E. coli, K. pneumoniae, S. aureus	305
28	Elaeagnus umbellata		Spherical; 20–100 nm	E. coli, S. aureus	306
29	Sida cordifolia	Whole plant	Spherical; 3–8 nm	B. subtilis, S. aureus, E. coli, K. pneumonia, A. hydrophila, P. fluorescence, F. branchiophilum, E. tarda, Y. ruckeri	307
30	Sida acuta	Whole plant	Spherical; 14.9 nm	E. coli, S. aureus, S. faecalis	308
31	Rheum ribes	Whole plant	Spherical; $3.32 \pm 0.58$ nm	S. pyogenes, S. aureus, S. typhimurium, E. coli	309
32	Blumea eriantha	Whole plant	Spherical; 50 nm	B. cereus, B. subtilis, S. aureus, E. coli	310
33	Arnicae anthodium	Whole plant	Irregular; 90-118 nm	S. aureus, E. coli, P. aeruginosa, C. albicans	311
34	Salacia chinensis	Whole plant	Irregular; 20–80 nm	S. aureus, P. aeruginosa, E. coli, S. typhi	312
35	Ferocactus echidne	Whole plant	Elliptical; 20–60 nm	E. coli, S. aureus, C. Albicans	313
36	Rosa indica	Petals	Spherical; 23.52–60.83 nm	S. mutans, E. coli, K. pneumoniae, E. faecalis	43
37	Hibiscus rosa-sinensis	Petals	Cube; 76.25 $\pm$ 0.17 nm	V. cholerae, E. coli, K. pneumoniae, S. aureus	314
38	Clitoria ternatea L.	Petals	Spherical and flat plate; 35-80 nm	S. aureus, Shigella sp.	39
39	Euphorbia antiquorum L.	Latex	Spherical; 10–50 nm	E. coli, K. pneumoniae, P. mirabilis, V. cholerae, E. faecalis	315
40	Calotropis gigantea L.	Latex	Spherical; 5–30 nm	Bacillus, Enterococci, Shigella, P. aeruginosa, K. pneumonia, Staphylococcus, E. coli	316
41	Cocoa	Pod	Spherical; 4–32 nm	E. coli, K. pneumoniae, S. pyogenes, S. aureus P. aeruginosa, A. flavus, A. fumigatus and A. niger	317

Table 4 (Contd.)

No.	Plants	Plant part	Shape and size	Test microorganisms	Ref.
42	Cola nitida	Pod	Spherical; 12–80 nm	K. granulomatis, P. aeruginosa, E. coli, S. aureus, A. niger, A. flavus, A. fumigatus	318
43 44	Taxus yunnanensis Chlorophytum borivilianum L.	Callus Callus	Spherical; 6.4–27.2 nm Spherical; 52.0 nm	E. coli, S. aureus, S. paratyphi, B. subtilis P. aeruginosa, B. subtilis, Methicillin- resistant Escherichia coli, S. aureus, C. albicans	319 320

incubation time. The authors reported that the synthesized AgNPs displayed synergistic effects with the antibiotic levofloxacin.288 Citrus maxima peel extract was utilized as both reducing and capping agent for the green synthesis of AgNPs with size ranging from 4-11 nm. Bactericidal activity of the synthesized AgNPs was tested against E. coli and S. aureus and found that the Citrus maxima peel extract mediated AgNPs showed great inhibitory action against both the bacterial strain. Furthermore, bactericidal activity was also examined against plant pathogens such as F. oxysporum and V. dahlia, showed excellent inhibitory action against both the pathogens.291 Besides, Punica granatum, 292 Citrus × clementine, 293 and Solanum melongena L.294 peel extracts were exploited for the green synthesis of AgNPs and tested its bactericidal activity against various bacterial strains. The results revealed that the synthesized AgNPs displayed great cell wall damage of both Grampositive and Gram-negative bacteria. Interestingly, AgNPs derived from Punica granatum peel extract showed high ZOI against S. aureus (16.5 mm) compared to E. coli (15.5 mm).292 Microwave irradiation technique has been utilized for the green synthesis of AgNPs from Solanum melongena L. peel extract to increase the rate of bioreduction process of AgNO<sub>3</sub>. Moreover, the size and the shape of the nanoparticles generated via this process is 92.4 nm and spherical.294 In another work, Dutta et al.295 have reported the green synthesis of AgNPs using Citrus limetta peel extract as both reducing and capping agent. Investigation of bactericidal activity of the synthesized AgNPs against various pathogens such as C. albicans, C. glabrata, C. parapsilosis, C tropicalis, M. luteus, S. mutans, S. epidermidis, S. aureus, E. coli revealed that the Citrus limetta peel extract mediated AgNPs have cell disruption potential and hence can be used in pharmaceutical industries. Furthermore, the antifungal activity test of the synthesized AgNPs against Candida species revealed that the nanoparticle has the ability of cell membrane distortion. The effect of AgNPs on micromorphological changes of C. albicans was clearly visible and found that AgNPs induces the cell blebs and a thick exudate deposition around the cell that demonstrate the leakage of intercellular components. From the results, the authors reported that Citrus limetta peel extract mediated AgNPs have excellent antifungal activity.

#### 3.11 From tube/bulb

A diverse tube/bulb extract of plants are reported for the green synthesis of AgNPs (Table 4, entries 18–22). Recently, onion

(Allium cepa) extract was employed as a reducing as well as a capping agent for the biosynthesis of spherical AgNPs with size ranging from 10-23 nm. The authors reported that the synthesized AgNPs have excellent antimicrobial activity against B. subtilis, B. cereus, B. licheniformis, S. aureus, S. mutans, E. coli, K. pneumoniae, S. typhimurium, P. aeruginosa, P. vulgaris, S. marcescens, C. albicans.296 A wide variety of tube/bulb extract such as Sunroot tuber,297 Dioscorea alata298 and Crocus haussknechtii Bois299 were utilized for the green synthesis of AgNPs and examined the bactericidal activity of the synthesized nanoparticles against both Gram-positive and Gram-negative bacterial strains. It is reported that, spherical AgNPs with size range 10-25 nm was obtained using 20 mM AgNO<sub>3</sub>, 0.5 mL Crocus haussknechtii Bois extract at pH 7 and temperature of 75 °C.<sup>299</sup> An obvious result was obtained in case of *Dioscorea* alata mediated AgNPs, where it is observed that the ZOI for E. coli is greater than S. aureus. 298 In another work, aqueous extract of Allium sativum was employed as both reducing and capping agent for the synthesis of AgNPs. To investigate the antimicrobial activity, the authors have applied the synthesized NPs to the pathogenic bacteria such as P. aeruginosa and B. licheniformis and found that the nanoparticle has the cell permeable ability and hence can be used in biomedical applications to make antimicrobial drug.300

#### 3.12 From the whole plant

The exploitation of plant extract in the biosynthesis of AgNPs is an important field in nanobiotechnology. To date, numerous literatures are available for the green synthesis of AgNPs using plant extract (Table 4, entries 23-35). Sargassum muticum,301 Brassica oleracea L. (Broccoli), 302 Vernonia cinerea L., 303 Artemisia marschalliana<sup>304</sup> and Linum usitatissimum L.<sup>305</sup> plant extracts were exploited for the green synthesis of AgNPs. The resulted nanoparticles were utilized against both Gram-negative and Gram-positive bacterial strains to investigate its bactericidal activity, and it is observed that the plant extract mediated AgNPs are capable of bacterial cell damage at a very low concentration. Different concentration of AgNPs derived from Artemisia marschalliana plant extract was used against S. aureus, B. cereus, A. baumannii, and P. aeruginosa and found that the ZOI is highest for S. aureus unlike other plant extract mediated AgNPs. Therefore, the authors claimed that the photosynthesized AgNPs from Artemisia marschalliana plant extract can compete with the commercial antibiotics.304 In comparison of the bactericidal activity of AgNPs derived from callus extract and

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whole plant extract revealed that callus extract mediated AgNPs are smaller in size and thus displayed high bactericidal activity.305 Ali et al.306 have reported shape/size-selective green synthesis of AgNPs using Elaeagnus umbellate extract and treated against various bacterial pathogens such as E. coli and S. aureus to examine its bactericidal activity. The results showed that the Elaeagnus umbellate extract mediated AgNPs can effectively damage the cell membranes as well as releases cellular matrix and hence can be used in pharmaceutics. To further investigate the morphological changes of the bacteria S. aureus and E. coli, SEM analysis was performed, where the images displayed that before AgNPs treatment the cell membranes of the two bacteria remain intake and have a regular morphology. However, after the treatment of AgNPs, no definite cell wall was observed, and membrane disruption occurs. Besides, antimicrobial activity of AgNPs derived from plant extract of Sida cordifolia,307 Sida acut,308 Rheum ribes,309 Blumea eriantha310 and Arnicae anthodium311 were examined against various bacterial strains. The small size of AgNPs (50 nm) derived from Blumea eriantha plant extract displayed excellent growth inhibition of bacterial cell as it provides a high surface area to the pathogens and thus effects more compared to the larger AgNPs.310 Similarly, Salacia chinensis extract was employed for the green synthesis of AgNPs and examine their antimicrobial activity against S. aureus, P. aeruginosa, E. coli, S. typhi. The antimicrobial activity testing results revealed that the Salacia chinensis extract mediated AgNPs showed high inhibition activity against S. aureus and P. aeruginosa. However, it showed minimum inhibition activity against E. coli and S. typhi.312 Plant extract of Ferocactus echidne was utilized for the green synthesis of AgNPs. The synthesized nanoparticles were utilized against various human pathogens and found that the nanoparticle is active against both Gram-positive and Gramnegative bacteria.313

#### 3.13 From petals, latex, pod and callus

Different parts of plants such as petals (Table 4, entries 36–38), latex (entries 39 and 40), pod (entries 41 and 42) and callus (entries 43 and 44) in the form of their aqueous/alcoholic extract have been utilized for the green synthesis of AgNPs. It is reported that Rosa indica43 and Hibiscus rosa-sinensis314 petal extract were utilized for the green synthesis of spherical AgNPs. Both the petal extract mediated AgNPs displayed good bactericidal activity against Gram-negative bacteria compared to Gram-positive bacteria. 43,314 The size of the synthesized nanoparticle plays a vital role in the bactericidal activity test. Smaller the size of the nanoparticle greater is the surface available to adhere to the microorganisms, which led to the change in the Physicochemical properties of the bacterial cell and finally led to bacterial cell damage.314 Vanaraj et al.39 reported the green synthesis of AgNPs by using Clitoria ternatea L. extract as a bioreducing agent and examined their antimicrobial activity against S. aureus and Shigella sp. and found that the synthesized AgNPs can effectively disrupt the cell membranes of both the bacterial pathogens.

Latex extract of Euphorbia antiquorum L. was employed for the green synthesis of AgNPs with size ranging from 10-50 nm.

Antimicrobial activity of the synthesized AgNPs was tested against various human pathogens such as E. coli, K. pneumoniae, P. mirabilis, V. cholera and E. faecalis and showed mild inhibition activity against all mentioned pathogens.315 Similarly, antimicrobial activity of spherical AgNPs derived from Calotropis gigantea L. against various human pathogens has been investigated and displayed remarkable activity against both Gram-positive and Gram-negative bacteria.316 Pod extract of Cocoa was utilized for the biosynthesis of AgNPs. The synthesized nanoparticles showed great inhibition against E. coli and K. pneumonia. Moreover, the nanoparticle improves the activity of cefuroxime and ampicillin synergistically.317 In addition, Lateef et al.318 have reported the green synthesis of AgNPs using pod extract of Cola nitida as a reducing as well as capping and stabilizing agent. Antimicrobial activity of the synthesized AgNPs revealed that at different AgNPs concentration ranging from 50-150 μg mL<sup>-1</sup> showed great inhibition activity against K. granulomatis, P. aeruginosa, and E. coli. Besides, incorporation of 5  $\mu$ g mL<sup>-1</sup> of pod extract of *Cola nitida* mediated AgNPs into the paint completely inhibits the growth of bacteria such as S. aureus, E. coli, P. aeruginosa, A. niger, A. flavus and A. fumigatus and hence can be utilized in paint manufacture industries and biomedical.

Recently, callus extract of Taxus yunnanensis has been employed as a reducing and stabilizing agent for the green synthesis of AgNPs and examined their bactericidal activity against both Gram-positive and Gram-negative bacteria. The bactericidal activity test of the synthesized AgNPs revealed that the inhibition effect is more pronounced in case of Grampositive compared to Gram-negative bacteria. Therefore, callus extract of Taxus yunnanensis mediated AgNPs can be used in antibiotic therapeutics, an alternative to the antibacterial drug.<sup>319</sup> Spherical and well-dispersed AgNPs were also prepared from callus extract of Chlorophytum borivilianum L. It is reported that the synthesized nanoparticle can effectively inhibit almost all kinds of human pathogens.320

# Mechanism of antibacterial inhibition by bioinspired AgNPs

The actual mode and reactive species, whether AgNPs321-323 or the released Ag<sup>+</sup>, 80 in the bactericidal activity of AgNPs is not well established to date and is still a topic of hot debate. However, most of the recent studies revealed that released Ag<sup>+</sup>, not the actual AgNPs, is possible the antimicrobial agent that causes cell damage and consequent death.11,324,325 Several pathways have been that proposed for the bactericidal activity of AgNPs which include the generation of reactive oxygen species,326 free radicals derived from the surface of AgNPs,327 silver ion stress,328 coating agents,329 interactions with the bacterial cell that leads to depletion of intracellular ATP level322 and damage in respiratory enzymes.330

The possible mechanism for the antibiotic activity of AgNPs is displayed in Fig. 9. It is reported that the smaller the size of AgNPs greater is the bactericidal activity as it provides a greater surface to the bacterial membrane. The interaction between the Review RSC Advances

positively charged Ag ion with the negatively charged cell membranes led to the disruption of the cell morphology and hence cell leakage occurred, resulting in cell death. Besides, AgNPs bind strongly with phosphorus and sulfur of the extracellular and intracellular membrane proteins, thus affects the cell replication, respiration and finally, the lifetime of the cell. Apart from that, AgNPs can also bind with the thiol and amino groups of membrane protein and led to the formation of reactive oxygen species (ROS), which inhibits the cell respiration. The excellent bactericidal activity of AgNPs can be attributed to the interaction with the plasma membrane and peptidoglycan cell wall of the bacterial strain.<sup>331</sup> It has also been suggested that the interaction of AgNPs with cell wall increases the membrane permeability by forming pores or pits and thereby causing the death of bacteria.<sup>332,333</sup>

#### Conclusion and future outlook

Taking into account the many benefits of green synthesis of AgNPs using plant extracts and their excellent antimicrobial activities as bare or in conjugation with antibiotic drugs, there is no doubt that this research field will continue to attract much interest in recent years. Here different biogenic methods for the synthesis of AgNPs using phytochemicals, nontoxic, inexpensive, and eco-friendly route has been comprehensively reviewed. The antimicrobial susceptibility of the produced AgNPs against several pathogenic microbes has also been highlighted. Although the rapid and green synthetic methods using plant extracts have shown great potential in AgNPs, understanding the mechanism by which phytochemicals of these plants are involved in the synthesis and the mode of antimicrobial inhibition are still not fully understood. In addition, controlling the shape of biosynthesized AgNPs, which have many positive effects on its activities, remained largely unanswered till today although chemical methods are already well-known for shapecontrolled synthesis. This problem is potentially due to the large number of different phytochemicals present in the plant extract, making it difficult for a systematic control of the interaction with the produced AgNPs. Hence, better understanding of each phytochemical, quantities and their

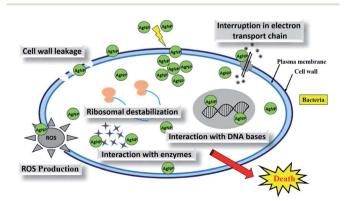


Fig. 9 Possible mechanism for the bactericidal activity of AgNPs. This figure has been reproduced from ref. <sup>331</sup> with permission from Elsevier, copyright 2004.

interaction will pave the way for shape-selective synthesis of biogenic NPs. In general, the smaller the NPs better its antimicrobial activities due to the increase in surface are that are in contact with the microbial cell. 118,141 Of the same size range, antimicrobial activities of AgNPs are in the order; triangular > pentagonal, hexagonal, cubic, nano-rod > spherical. Triangular one showed the highest activity mainly due to better edge fitting due to sharp edge and predominant stable (1 1 1) facet. 109,112,113 Hexagonal, cubic, nano-rod have bend edge, which might have reduce their efficacy towards microbes as compared to triangular shape NPs. 334 whereas spherical shape NPs with no sharp edge and predominantly (1 0 0) facets showed least antimicrobial effects. 113

Several authors revealed that Gram-positive bacteria (*e.g. S. aureus*), due to their thick cell wall of peptidoglycan layer (~20–80 nm thick), are less susceptible to AgNPs than Gram-negative bacteria (*e.g. E. coli*) with cell wall consisting of lipopolysaccharides at the exterior, followed underneath by layer of peptidoglycan (~7–8 nm).<sup>75,102,103,107,108,118,130,166,236</sup> However, this is not the case everywhere.<sup>108,154,194,228,230,280</sup> In the light of this, one must look into the role of lipopolysaccharides in Gram-negative that might have acted as a shield against some AgNPs and also the synergetic effect of AgNPs and biomolecules that act as a capping that might have alter the mode of interaction of NPs with the cell wall. Hence understanding the underlying mechanism of the interaction is still a challenge.

The antimicrobial efficacy of AgNPs can be greatly enhance by its synergistic interaction with many well-known antibiotic drugs. <sup>10,11,77,80,104,127,183,261,288,317</sup> This opens a new and exciting opportunity in combating numerous newly evolved highly infectious multi drug-resistant microbes. Hence, this research field has become a 'hot' topic in recent years although it is in its infancy. To have a better insight, understanding the mechanism of interaction of the AgNPs with drugs and the alteration in the mode of attack due to the synergetic interaction towards the microbes needs to be well understood and validated experimentally.

The successful green synthesis of AgNPs and evaluation, understanding the antimicrobial activities is a complex process till today although this research field has been explored several decades. However, looking at the literature we can draw several assumptions which potentially provide us AgNPs with high antimicrobial activities. Hence, knowing the complexity of the research on the green synthesis and antimicrobial activity of AgNPs, the below points are worth considered during AgNPs synthesis:

#### (1) Chemical composition of the plant extract

It is believed that the oxidation of different biomolecules such as flavonoids, ketones, aldehydes, tannins, carboxylic acids, phenolic and the protein of the plant are mainly responsible for the reduction of Ag<sup>+</sup> to Ag<sup>0</sup>. In addition, the stability and size of the produced AgNPs depends on the biomolecules acting as a capping agent.<sup>138</sup> Hence, one must first investigate the biomolecules present in the plant extract and its capping efficacy for successful synthesis of AgNPs. In general, greater the

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capping activity greater the stability and lesser is the average particle size of NPs. 335,336 However, this is not true in some case.337,338 Hence, specific interaction of the biomolecules and the formed NPs also need attention on case to case basis.

#### (2) Concentration of the plant extract

The shape and size of the synthesized AgNPs depend on the concentration of the plant extract used. At dilute concentration, formation of the NPs may not even take places; hence one need to investigate carefully. Formation of AgNPs is usually accompanied by colour change and prominent UV-Vis absorption at around 430 cm<sup>-1</sup>. Increase in extract concentration leads to formation a large number of NPs to a certain level. 76 However, while desired concentration of extract can afford a highly disperse AgNPs with high antimicrobial activity, high concentration of extract often leads to agglomeration and large NPs, as excess reducing agents potential caused secondary reduction process on the surface of the preformed nuclei.339

#### (3) Concentration of AgNO<sub>3</sub>

The number of AgNPs increased with the increase in AgNO<sub>3</sub> concentration up to the level where all the AgNO<sub>3</sub> salt are consumed i.e. all Ag<sup>+</sup> are reduced to Ag<sup>0</sup>, which can be easily monitored by increasing intensities in UV-Vis spectroscopy. Once all the AgNO<sub>3</sub> are consumed, an equilibrium will be reach. Hence one need to see the balance between the AgNO3 and the amount of reducing agent present in the extract.76,235,261

#### (4) Extraction solvent

Different biochemicals in the plant has different level of solubility in solvents; hence the successful extraction of the desired biochemicals for synthesis of AgNPs largely depends on the extraction solvent used. Phenolic compounds are known to be highly soluble in ethanol, methanol as well as their mixture with water (ethanol-water or methanol-water). 43,185,199,200,204 Hence, these are a solvent of choice for extraction along with pure water which is most used.

#### (5) Extraction time and temperature

Another important factor to consider for the successful synthesis of biogenic AgNPs is the extraction temperature. It is well known that solubility of biochemicals increased with increase in extraction temperature and time. Hence, more chemical will be extracted at a higher temperature which will make it a strong reducing agent. But there is a possibility of extracting a non-reactive biochemicals or decomposition of biochemicals at a long time at higher temperature. 185,187

#### (6) pH

pH can change the electrical charges of biomolecules in the plant extract that might have affect the nature of their capping and stabilizing affinity and subsequently the growth of NPs. Increased in pH usually resulted in the increase rate of formation as well as promotes homogeneous distribution of size of NPs. 149,340 However, under acidic condition slow formation and

agglomeration took place resulting in larger NPs. 151,152,341 At the same time, high pH can lead to precipitation of AgOH which is undesirable.150 Hence, neutral pH (7) is highly recommended if an external buffer is used.

#### (7) Reaction time

The size of the NPs are reported to increases with time<sup>74,111,163</sup> as indicated by a red-shift in UV-Vis. Spectrometer data. Hence vigilant monitoring of the reaction to get a stable small size NPs is critical.

#### (8) Reaction temperature

High temperature is usually required to achieve complete reduction of AgNO<sub>3</sub> to AgNPs using chemical route, 337,342 although from economic and green chemistry prospective, RT reaction is the best choice. However, when it comes to green NPs synthesis, RT process, despite there are some exceptions, usually afford spherical shape NPs which are less susceptible to microbes as mentioned earlier. In the meantime, synthesis of different shapes of NPs for specific purpose is highly desirable. Literature review revealed that formation of cubic, 334,343 pentagonal, hexagonal,111 triangular, rod-shape nanowire343 AgNPs happen usually above RT, although some other parameters such as capping agents and stabilizers concentration needs to be taken care. Hence, in addition to increasing the speed of reaction and decreasing the size of NPs with temperature, one must consider the reaction temperature to produce NPs with different shape for a specific purpose, particularly as a potent antimicrobial.

#### Conflicts of interest

None to declare.

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