REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1 (2024)
http://www.veterinaria.org
Article Received: 23/03/2024 Revised: 07/04/2024 Accepted: 20/04/2024

Synthesis of Silver Nanoparticles Using *Terminalia Chebula* Plant Extract and Evaluation of Its Antimicrobial Potential with Selected Antibiotics

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ABSTRACT
The expansion of the biological production of nanoparticles expending microorganisms or herbal extracts the stage a central person in the arena of nanotechnology as it is ecologically responsive and prepares not encompass any injurious elements. In this training, the amalgamation of silver nanoparticles expending the shrubberies quotation of *Terminalia Chebula*. The created nanoparticles were considered consuming UV-vis spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM). The XRD investigation displays that the manufactured silver nanoparticles are of face-centered cubic building. Well-dispersed silver nanoparticles with a ballpark size of 4 nm were pragmatic in the TEM image. Silver nanoparticles assessed antimicrobial activity its displayed first-rate potency touching usual drugs. The request of the green synthesized nanoparticles can be charity in several pitches such as makeups, foods, and medication.

KEYWORD: *Terminalia Chebula*, AgNps, Antibiotics, Antimicrobial Activity, XRD, FTIR, TEM

INTRODUCTION

The growth of deadliness free metal nanoparticles has developed a prodigious contest in current times. The chief contest in this principal extent is the enlargement of the tradition of conservational outgoing supplies in the cohort of metal-nanoparticles. Numerous chemical/physical approaches have been working to concoct silver nanoparticles with changed dimensions and shapes, such as UV radiation, microware-irradiation, photochemical method and electrochemical method.[1-9] Though, maximum of the testified methods comprise additional than one phase, high vigor prerequisite, little substantial alterations, exertion in sanitization, and dangerous chemicals. The chemical separation of nanoparticles may chief to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects fashionable its submission. The separation of nanoparticles by green method can possibly abolish this problem. Consequently, there is an imperative need to progress a green development of nanoparticle synthesis.[10,11]

Green synthesis approaches retaining either biological microbes or plant cuttings have emerged as an unassuming and substitute to chemical amalgamation. Green amalgamation delivers developments finished chemical approaches as it is atmosphere outgoing, cost active, and effortlessly mounted up for great scale fusion. The amalgamation of nanoparticles by expending plant extracts can be beneficial over additional biological progressions since it abolishes the extravagant development of continuing cell beliefs and can be befittingly scaled up for huge scale construction beneath non-aseptic situations.[12]

Silver-nanoparticles are well identified as one of the record common antimicrobial materials in the arena of biology and drug due to their stout biocidal result alongside microbial types, which has been charity for eras to avoid and treat various sicknesses, most especially pollutions.[13] Silver-nanoparticles also testified to hold anti-viral,[14] anti-cancer,[15] anti-oxidant,[16] and anti-microbial potency.[17] Newly, the progress of silver nanoparticles is mounting. They are currently charity as part of tailored, and transplant varnishes.[18]

T. Chebula has been extensively charity as herb in Ayurveda, Unani and Homoeopathic drug likewise it is a middle of magnetism in current medicine. *T. Chebula* are testified to have high antioxidant belongings and the capability to diminution the lower blood pressure, cholesterol, prevent cardiovascular illnesses and increase the regular flora in our intestinal territory.[19-21] In the present-day effort, we have projected a green biological technique for creating Ag nanoparticles consuming *T. Chebula* extract from *Camellia sinensis* as dropping and plugging negotiators. To the greatest of our information, this education is original and offerings a unassuming methodology to manufacture Ag-nanoparticles proficiently at room temperature.[22-25]

Hereafter, in this work we ponder the antimicrobial potency of exact antibiotics freezing with *T. Chebula*plant extract in dissimilarity to particular plant pathogenic microbes and fungi. This schoolwork also investigates the chemical

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conformations of novel composite twisted or not by expending UV-vis spectroscopy, X-ray diffraction, TEM and XRD analysis.

MATERIAL AND METHOD

Collection of Plant Material

Garden-fresh *T. Chebula* were developed from dissimilar area of Northern Gujarat. The detached mockups were arranged to confiscate bad eminence, sodden in tap water, eroded and rinsed underneath seriatim water. They remained cut, dried, pulverized, warehoused in antibacterial complaint and secondhand for extra studentships.

Synthesis of Silver Nanoparticles

The *T. Chebula* extract was willing by evaluating 10 g of *T. Chebula* in 500 mL tumbler along with 100 mL of distilled water and preserved at 60°C for 10 min before emptying it. The solution was sieved by 0.45 µm Milipore sheath filter and shadowed by 0.2 µm Millipore casing riddle. For synthesis of Ag-nanoparticles, 100 mL of $AgNO_3$ (1 mM) was countered with 12 mL of the *T. Chebula* extract in Erlenmeyer flask at room temperature. Some color variations of the explanation were pragmatic.

Antimicrobial evaluation

Agar-well-diffusion method elective by Arodiya *et al.* charity to appraise the antimicrobial potency of *T. Chebula* extracts, Antibiotics, and their recipes. Agar broadcasting was equipped by consuming Muller Hinton Agar. The agar plate external was injected by scattering the particular bacteriological (*S. aureus, B. subtilis, P. aeruginosa, E. coli, A. niger* and *C. albicans*) completed the complete agar shallow. Then, wells, a distance of 8 mm were stamped with an antiseptic cork bit and 50 µL of the confirmed solution at looked-for meditations announced into the well. Then, agar plates were raised at 37 °C for 24 hrs. The zone of hindering was charity to direct the antimicrobial potency in mm.

RESULT AND DISCUSSION

The color change was renowned by graphic thought in the Erlenmeyer-flask which comprises $AgNO_3$ solution with *T. Chebula* extract. The color of the $AgNO_3$/ *T. Chebula* extract solution different from neutral to light chocolate after 5 min and ultimately to dark chocolate (Fig. 1). This color alteration designates the development of Ag-nanoparticles in the explanation. Tea extract deprived of $AgNO_3$ did not display any color variations. The development of Ag-nanoparticles was further established by consuming UV-visible spectroscopy, X-ray diffraction, Fourier-Transform infrared spectroscopy and transmission electron microscopy.

Figure 1. Aqueous solution of $AgNO_3$ with *T. Chebula* extract (**A**) before adding the *T. Chebula* extract and (**B**) after addition of *T. Chebula* extract at 5 min.

Fig. 2 displays XRD designs for Ag-nanoparticles manufactured by *T. Chebula* extract. Five chief characteristic spreading summits for Ag were observed at 2θ = 37.3, 45.5, 63.5, 76.8 and 82.3, which correspond to the 111, 200, 220, 311, and 222 crystallographic aircrafts of face-centered cubic (fcc) Ag gemstones, respectively. No summits as of any other phase remained pragmatic viewing that solitary phase Ag with cubic assembly nanoparticles have been attained straight.

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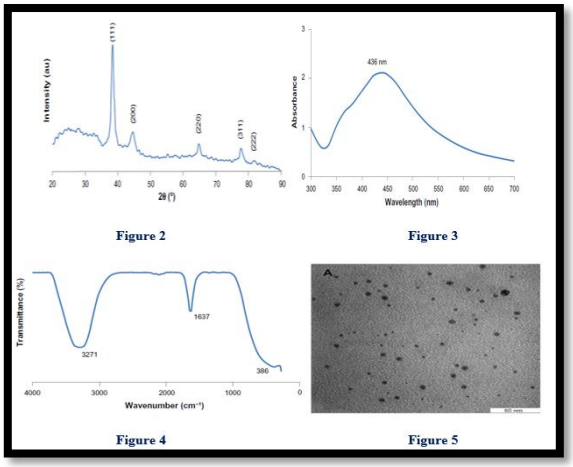

Figure 2. XRD patterns of Ag nanoparticles, **Figure 3.** XRUV-Vis spectrum of Ag nanoparticles., **Figure 4.** FTIR spectrum of Ag nanoparticles., **Figure 5.** TEM image of Ag nanoparticles.

In overall, the breadth of XRD peaks is associated to crystallite size. Debye-Scherrer calculation was cast-off to govern usual crystallite length from half thickness of the spreading crests: $D = (k\lambda)/(\beta \cos \theta)$, where D is unkind crystallite size of the ash, λ is the wavelength of Cukα, β is the occupied thickness at half-maximum, θ is the Bragg spreading approach and k is a relentless. The plane was elected to estimate crystalline size. Since Debye-Scherrer calculation, the normal crystallite size of Ag-nanoparticles created is originate to be 3.45 nm.

Fig. 3 displays the UV-vis fascination spectrum of the manufactured Ag-nanoparticles. Silver nanoparticles have allowed electrons, which give external Plasmon character absorption band, due to the mutual tremor of electrons of Ag-nanoparticles in character with bright wave. A broad interest peak was pragmatic at 436 nm, which is a distinctive mob for the Ag. No additional crowning was pragmatic in the gamut which authorizes that the created harvests are Ag only.

FTIR dimension was accepted out to find the conceivable biomolecules answerable for topping and dropping agent for the Ag nanoparticles produced by cm^{-1} quotation. Three understandable electromagnetic gangs are pragmatic at 3271 cm^{-1}, 1637 cm^{-1} and 386 cm^{-1} (Fig. 4). The forceful wide band at 3271 cm^{-1} is due to N-H and O-H stretching approach in the relation of the proteins. The middle powerful band at 1637 cm^{-1} ascends from the >C=O stretching method in amine group which is frequently originate in the protein, representing the occurrence of proteins as stopping agent for Ag nanoparticles which surges the firmness of the nanoparticles created. On the added hand, the strong and broad topmost at 386 cm^{-1} connected to the Ag metal.

Transmission-electron-microscopy (TEM) has been working to characterize the size, outline and morphology of created silver nanoparticles. The TEM image of silver nanoparticles is displayed in Fig. 5. Since the double, it is manifest that the morphology of Ag nanoparticles is globular which is in covenant with the profile of SPR band in the UV-vis band.

The globally benign Ag nanoparticles mixture progression have probable bids in verious fields. Silver nanoparticles can be pragmatic in varnish for planetary energy, bio-labelling, nutrition packing, antimicrobial manager and medicine sending. Some trainings on the procedure of metal nanoparticles in the water mesh have been passed out owed to its antibacterial and as pesticide subtraction belongings. Also that, silver nanoparticles show an imperative role in the therapeutic area. Silver nanoparticles entertainment as biomarker in uncovering of primary finding and healing nursing such as the finding of cancer for cancer handling and primary finding for Alzheimer's illness.

Antimicrobial Evaluation

Methanol/ethanol/acetone extracts of crumpled ***T. chebula*** bark were confirmed for their antibacterial clothes on their own and in amalgamation with particular antibiotics (Amoxicillin, Ceftazidime, Ciprofloxacin and Erythromycin).

In incongruity of tested bacterial kind, all three passages exhibit effective antibacterial potency.

Acetone extract displays zone of inhibition amongst 12 to 20 mm for precise bacterial land for 1% w/v (25 µg/ml) consideration ethanol citation displays zone of shyness among 10 to 16 mm for precise bacterial nation. Methanol extract display zone of embarrassment between 08 to 14 mm for accurate bacterial strain.**(Table 1)** all three citations displayed prevailing antibacterial potency against *P. aeruginosa* and *E. coli* species.

Amoxicillin expressively summary the increasing of *S. aureus* and *B. subtilis*, its assortment with all three quotations also exhibitions remarkable growth nervousness in inconsistency of these two types midst all observed bacteria. An consolidation of methanol extract with amoxicillin exhibitions 43 mm, 42 mm, 32 mm, and 31 mm of ZOI against *S. aureus, B. subtilis, P. aeruginosa,* and *E. coli*, independently, at 1% w/v meditation. The mixture of amoxicillin with methanol and ethanol etchings also displayed higher potency than with acetone extract, equivalent to pure quotations**(Table 2)**.

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Ciprofloxacin when association with extract it is showed that the mixture have developed fallback consequence opposing to all stain. All three solvent extract and its consortium displays synergic consequence in inconsistency of particular bacterial principles **(Table 3)**.

When extract communal with Ceftidizime it has good conflict potential in illogicality of all four microbial philosophy. Pure extract and combination has respectable pugnacious possible. As per **Table 4** methanol, ethanol and acetone exctract and its incorporation have respectable synergic product alongside bacterial philosophy. 1% w/v explanation have moral struggle probable against *E. coli* almost 17 mm to 23 mm.

Erythromycin and its assortments with all three extracts show higher potency in contradiction of *S. aureus* and *B. subtilis* and lesser potency against *P. aeruginosa*. Alliance of erythromycin with acetone extract divulged 36 mm, 32 mm, 27 mm and 31 mm whereas ethanol extract displayed 38 mm, 36 mm, 31 mm, and 32 mm, and with methanol extract photograph 35 mm, 34 mm, 28 mm, and 31 mm of ZOI alongside *S. aureus, B. subtilis, P. aeruginosa,* and *E. coli* similarly at 1000 µg/ml fascination **(Table 5)**.

Pure Silver nanoparticles acetone/ethanol/methanol extract displays zone of shyness amid 13 to 22 mm for exact bacterial country for 1% w/v (25 µg/ml) for accurate bacterial strain. **(Table 6)** all three extracts displayed powerful antibacterial potency against *E. coli* species.

Amoxicillin eloquently growth the increasing of all tensions its mixture with all three extracts also shows remarkable growth wariness in inconsistency of these all type midst all observed bacteria. An incorporation of silver nanoparticles with amoxicillin shows 48 mm, 45 mm, 35 mm, and 34 mm of ZOI alongside *S. aureus, B. subtilis, P. aeruginosa,* and *E. coli,* individually, at 1% w/v attention. The mixture of amoxicillin with silver nanoparticles also shown advanced potency than with corresponding to pure quotations Grouping of ciprofloxacin and silver nanoparticles when association with exhibited 45 mm, 43 mm, 36 mm, and 32 mm of ZOI to all stain. Ciprofloxacin and silver nanoparticles consortium shows synergic upshot in contradiction of separate infectious opinions.**(Table 7)**.

When silver nanoparticles association with Ceftidizime it has moral confrontation possible in inconsistency of all four microbial values. Pure extract and blend has respectable fighting possible. As per **Table 8** and its consolidation have good synergic product alongside bacterial nation. 1% w/v answer have upright skirmish likely against *E. coli* almost 22 mm to 27 mm. Erythromycin and silver nanoparticles parade higher potency in incongruity of *S. aureus* and *B. subtilis* and lesser potency alongside *P. aeruginosa.* Assemblage of erythromycin with 39 mm, 34 mm, 31 mm, and 34 mm of ZOI alongside *S. aureus, B. subtilis, P. aeruginosa,* and *E. coli* correspondingly at 1000 µg/ml captivation **(Table 8)**.

Table 1: Antibacterial activity of pure extract

Bacteria	**Acetone Extract**				**Ethanol Extract**				**Methanol Extract**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	17	16	15	12	15	14	14	10	13	12	11	10
B. subtilis	16	15	14	12	14	13	12	10	10	09	08	08
P. aeruginosa	15	14	12	10	13	12	10	09	12	10	09	08
E. coli	20	18	18	16	16	15	14	12	14	13	12	10

Table 2: Antibacterial activity of extract with Amoxicillin

Bacteria	**Acetone Extract + Amoxicillin**				**Ethanol Extract + Amoxicillin**				**Methanol Extract + Amoxicillin**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	41	40	39	38	42	40	39	38	43	42	41	40
B. subtilis	42	41	39	38	40	39	38	36	42	40	39	37
P. aeruginosa	32	30	31	29	30	29	28	26	32	31	29	27
E. coli	30	29	27	26	32	30	29	28	31	30	28	27

Table 3: Antibacterial activity of extract with Ciprofloxacin

Bacteria	**Acetone Extract + Ciprofloxacin**				**Ethanol Extract + Ciprofloxacin**				**Methanol Extract + Ciprofloxacin**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	42	40	39	37	39	38	36	36	40	39	37	36
B. subtilis	39	38	37	36	36	34	33	32	38	37	35	35
P. aeruginosa	34	33	31	30	30	29	27	26	32	31	30	28
E. coli	30	29	27	26	30	29	28	27	33	32	30	28

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Table 4: Antibacterial activity of extract with Ceftazidime

Bacteria	**Acetone Extract + Ceftazidime**				**Ethanol Extract + Ceftazidime**				**Methanol Extract + Ceftazidime**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	17	15	14	14	15	14	13	12	16	15	14	13
B. subtilis	15	14	13	12	14	14	13	13	11	10	09	08
P. aeruginosa	12	11	09	09	10	08	08	07	09	08	08	07
E. coli	23	22	20	19	19	18	17	17	20	19	18	17

Table 5: Antibacterial activity of extract with Erythromycin

Bacteria	**Acetone Extract + Erythromycin**				**Ethanol Extract + Erythromycin**				**Methanol Extract + Erythromycin**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	36	35	34	33	38	37	36	35	35	33	32	31
B. subtilis	32	31	29	28	36	35	34	32	34	33	31	30
P. aeruginosa	27	26	25	24	31	30	28	27	28	25	24	24
E. coli	31	30	28	27	32	31	30	28	31	29	28	27

Table 6: Antibacterial activity of pure Silver nanoparticles

Silver Nano Particles	**Conc. in µg/ml**	**Bacteria**			
		***S. aureus* (mm)**	***B. subtilis* (mm)**	***P. aeruginosa* (mm)**	***E. coli* (mm)**
Pure Silver Nano particles	1000	20	18	17	22
	500	19	16	15	20
	250	18	15	14	19
	125	15	14	13	18

Table 7: Antibacterial activity of Silver nanoparticles with Amoxicillin and Ciprofloxacin

Bacteria	**Silver nanoparticles + Amoxicillin**				**Silver nanoparticles + Ciprofloxacin**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125
S. aureus	48	45	43	39	45	42	40	35
B. subtilis	45	44	40	38	43	40	38	36
P. aeruginosa	35	34	32	30	36	34	32	29
E. coli	34	31	29	27	32	30	28	25

Table 8: Antibacterial activity of Silver nanoparticles with Ceftazidime and Erythromycin

Bacteria	**Silver nanoparticles + Ceftazidime**				**Silver nanoparticles + Erythromycin**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125
S. aureus	21	20	18	17	39	38	35	32
B. subtilis	18	17	14	12	34	31	30	28
P. aeruginosa	14	13	11	09	31	30	28	25
E. coli	27	25	24	22	34	32	28	24

Antifungal Evaluation

The antifungal potency of methanol, ethanol, and acetone extract of *T. chebula* and their blend with two antifungal antibiotics was appraised.

Pure extract displayed antifungal potency with deference two fungi. Acetone exctract display 17 mm and 16 mm zone of hang-up against *A. niger* and *C. albicans* singly at 1000 µg/ml meditation **(Table 9)**. Herbal plant methanol extract displays 14 mm and 11 mm ZOI alongside mentioned fungi and methanol exctract displayed 15 mm and 13 mm zone of shyness.

All three extracts displayed antifungal potency alongside *A. niger* and *C. albicans* **(Table 10)**. Amphotericin-B displayed active inhibition alongside both tested fungi, Merging all extracts with amphotericin-B displays active shyness, but methanol, ethanol and acetone all three extract displays a more operative synergistic upshot grouping with acetone extract displays 24 mm, 22 mm zone of inhibition, with ethanol citation displays 23 mm, 21 mm and with methanol quotation displays 23 mm and 22 mm ZOI against declared fungi.

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Fluconazole displayed minor potency compared to *A. niger* and sturdy potency display shyness alongside *C. albicans*. Blend with the extract of fluconazole dropped the shyness outcome of pure extract alongside both fungi **(Table 11)**. Blend displays 17 mm in both bacterial rinsing, zone of shyness with acetone quotation, 16 mm and 15 mm with ethanol quotation while with methanol extract it displays 15 mm and 16 mm zone of shyness. So all have resistance budding against revealed cherished molds.

Unalloyed herbal plant extract displayed antifungal potency with esteem two fungi. silver nanoparticles show 19 mm and 17 mm zone of shyness against *A. niger* and *C. albicans* individually at 1000 µg/ml meditation **(Table 12)**.

The silver nanoparticles displayed antifungal potency beside *A. niger* and *C. albicans*. Amphotericin-B displayed operative shyness alongside both tested fungi, coalescing silver nanoparticles with amphotericin-B displays effective shyness, silver nanoparticles displays a supplementary operative synergistic upshot recipe with *T. chebula* plant extract silver nanoparticles displays 16 mm, 15 mm ZOI against revealed fungi. Fluconazole displayed minor potency against *A. niger* and did not display shyness against *C. albicans*. Grouping with the extract of fluconazole dropped the inhibition outcome of untainted extract alongside both fungi **(Table 13)**. Amalgamation displays 07 mm and 05 mm ZOI. So all have fighting probable alongside mentioned respected fungi.

With depressing extract meditations, all of the combinations converted less effective alongside both of the tested fungi.

Table 9: Antifungal activity of pure extract

Fungus	**Acetone Extract**				**Ethanol Extract**				**Methanol Extract**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
A. niger	17	16	14	11	15	11	09	08	14	12	10	09
C. albicans	16	13	11	10	13	12	09	08	11	10	08	07

Table 10: Antifungal activity of extract with Amphotericin-B

Fungus	**Acetone Extract + Amphotericin-B**				**Ethanol Extract + Amphotericin-B**				**Methanol Extract + Amphotericin-B**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
A. niger	24	22	20	18	23	22	21	19	23	21	19	18
C. albicans	22	20	19	17	21	20	18	15	22	21	19	17

Table 11: Antifungal activity of extract with Fluconazole

Fungus	**Acetone Extract + Fluconazole**				**Ethanol Extract + Fluconazole**				**Methanol Extract + Fluconazole**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
A. niger	17	15	14	12	16	15	13	11	15	13	12	10
C. albicans	17	15	13	12	15	14	12	11	16	14	13	12

Table 12: Antifungal activity of pure Silver nanoparticles

Pure Silver Nano Particles	**Conc. in µg/ml**	**Fungus**	
		***A. niger* (mm)**	***C. albicans* (mm)**
Pure Silver Nano particles	1000	19	17
	500	17	15
	250	15	14
	125	14	12

Table 13: Antifungal activity of silver nanoparticles with Amphotericin-B and Fluconazole

Fungus	**Silver nanoparticles + Amphotericin-B**				**Silver nanoparticles + Fluconazole**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125
A. niger	27	25	24	21	22	20	19	17
C. albicans	26	24	22	20	20	19	18	15

CONCLUSIONS

The outcome of our study, green style for the amalgamation of Ag-nanoparticles using *T. chebula* extract. This is an unassuming, green and effective method to manufacture silver nanoparticles at room temperature deprived of consuming any destructive dropping mediators and any stopping or disbanding manager. It was determined that the green manufactured silver nanoparticles were serene of rotund particles which were exceedingly sparkling. The elements sizes were measured in the range from 2 to 10 nm. In totaling to dipping side properties by depressing antibiotic meditations, plant extracts can be exploited to growth the antibacterial potency of antibiotics. Subsequent satisfactory toxicological surveys, combinatorial chemistry can change the tradition of conformist medicines.

Acknowledgements

The authors are thankful to the Gokul Science College, Sidhpur for providing the necessary laboratory and library facility.

Conflict of Interest

The authors confirm that this article's content has no conflict of interest.

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