



PHYTOCHEMICAL SCREENING AND GREEN SYNTHESIS OF SELENIUM NANO MATERIALS USING *ALSTONIA SCHOLARIS* LEAVES AND THEIR ANTIMICROBIAL ACTIVITY

Sooraj Singh Kadam¹, A. Vinay Chandra², Arvind Kumar Sharma^{3*}

^{1,2}Research Scholar, P. K. University, Vill. Thanara, Karera, Shivpuri-473665 (M.P.), India.

³Quality Head, Advanced Environmental Testing & Research Lab, Gwalior-474011 (M.P.), India.

Article Received on 17 Feb. 2026,
Article Revised on 05 March 2026,
Article Published on 10 March 2026,

<https://doi.org/10.5281/zenodo.18957758>

*Corresponding Author

Arvind Kumar Sharma

Quality Head, Advanced
Environmental Testing & Research
Lab, Gwalior-474011 (M.P.), India.



How To Cite This Article: Sooraj Singh Kadam¹, A. Vinay Chandra², Arvind Kumar Sharma^{3*} (2026). Phytochemical Screening and Green Synthesis of Selenium Nano Materials Using *Alstonia Scholaris* Leaves and their Antimicrobial Activity. World Journal of Pharmacy and Pharmaceutical Sciences, 15(3), 1574–1582.

This work is licensed under Creative Commons Attribution 4.0 International license.

ABSTRACT

Alstonia scholaris is an evergreen tree commonly found in South East Asia. In traditional medicine pharmacological activities are attributed to the leaves and bark of this plant. The aim of this study is evaluate the presence of phytochemicals in *A. scholaris* leaves extracts and study their antimicrobial activities. Methanol and aqueous solvents were used in extractions with Soxhlet apparatus. The crude extracts were concentrated and screened for qualitative phytochemicals analysis, antibacterial and antifungal activity were measured. Aqueous and methanol extracts were used for green synthesis of nanomaterial AS-SeM and AS-SeAq, both showed significant antimicrobial activity and it was more pronounced against Gram positive than against Gram negative bacteria. Methanol and Aqueous extract of *A. scholaris* leaf showed activity against *Salmonella*, *E.coli*, *Klebsiella pneumonia* and *Candida albicans*. AS-SeM nano

material extract showed maximum activity against selected pathogenic microorganisms. In conclusion, *Alstonia scholaris* leaves extract are rich in phytochemicals and synthesized nano materials from leaves extracts of *A. scholaris* found with potential antimicrobial activities against pathogens.

KEYWORDS: *Alstonia scholaris*; Phytochemical; Antimicrobial; Antibacterial; Antifungus.

1. INTRODUCTION

Human pathogens are very significant microorganisms, which cause severe infections and finally death. In the 20th century, the most prominent therapeutic findings was the antimicrobial agents. However, efficacy of the discovered antibiotic were remained effective hardly for five decades, microbes is now start posing a great threat to mankind and emerged as global problem in the form of resistance against antibiotics (Peterson *et al.*, 2004). Microbes has developed resistance not only against all group of antibiotics but also against antivirals as well as antimycotics (Eloff *et al.*, 2000). This is indeed a relatively frightening state when noticed in 1990 that out of the 39.5 million of death in the developing countries, 9.2 million were estimated to have been caused by infectious and parasitic diseases and that 98% of death in children in developing countries resulted mostly from infectious diseases due to developed resistance to antibiotics (Murray *et al.*, 1997). Presently the Antibiotics are the easiest remedy due to indiscriminate use and abuse of antibiotics (Peterson *et al.*, 2004). Antibiotics used as a medicine alone for fast recoveries, the US Centre for Disease Control and Prevention estimates that approximately one-third of the 150 million prescriptions for antibiotics written unnecessarily each year. An appropriate solution or option will have to search to control this problem associated with drug resistance (Martini *et al.*, 1998).

Complementary therapies supported seasoning medications area unit the world's oldest style of medicine and up to date reports counsel that such therapies still fancy large quality, particularly in developing countries wherever most of the population doesn't have quick access to fashionable medication (Phukan and Phukan, 2014 and Tamilarasi and Ananthi 2012). The traditional Indian system of medication, Ayurveda, which suggests the science of life, is one among the world's oldest systems of medicines. Writing chiefly uses plant-based formulas developed through the experimentation and experiences of doctors for hundreds of years (Mukherjee and Wahile, 2006). In current world order, associate undiscovered reservoir of phytochemical info hidden in nature is quickly destroyed by deforestation and home ground loses. Ancient flavouring drugs is a crucial element of primary health care system in developing countries like Bharat. They're thought of to be safe, effective and cheap, that there's a worldwide trend for the revival of ancient flavouring drugs. Screening of healthful herbs employed by completely different ethnic teams or communities has currently become a possible supply for isolation of bioactive compound. *Alstonia scholaris* (L.)

Alstonia tree is a member of the *Apocynaceae* family which includes around 40 species that are spread throughout the tropical regions. The *Alstonia* mainly grows along damp river banks in the evergreen forests of most tropical countries and turns into giant trees. *A. scholaris*, *A. boonei*, *A. cogenesis*, and *A. neriifolia*, are the most prevalent species of plant *Alstonia* and significantly uses as drugs as anti-inflammatory, analgesic, ant diabetic, antimicrobial and anticancer because of its pharmacological importance.

Moreover about *A. scholaris*, this plant is also called with others names in Hindi as ‘*Shaitan ka jhar*’, ‘*Chitvan*’, Devil’s tree, Dita bark or milk wood, milky pine, and in English as white cheese, in Sanskrit and Tamils as ‘*Saptaparna*’ and *Pala* respectively. The leaves plant is arranged in a spiral of seven (‘*sapta*’ means seven and ‘*parna*’ or ‘*parni*’ means leaves in Sanskrit), so this plants is famous as ‘*Sapthaparna*’ or ‘*Saptaparni*’. The plant *Alstonia* was formally termed *Echites scholaris* by Linnaeus. *Echites pala* Buch-Ham ex Spreng, *Pala scholaris* (L.) Roberty, and *Tabernaemontana alternifolia* are additional synonyms for the plant (Chhajed et al., 2023). Prof. Charles Alston (1685–1760), keeper of the King’s garden at Holyrood, a renowned scientist, eminent botanist and Professor of Botany at Edinburgh University one of the few botanist who rejected the Linnaean taxonomic categorization and honoured as providing the generic name to this plant. In the past, this tree’s wood has historically been used to build wooden slates for Myanmarese school children in Southeast Asia, from this fact species name *Scholaris* is derived for the plant. *Alstonia scholaris* is a giant, evergreen tree that grows widely in tropical and subtropical regions of the world, including the western and southern regions, up to an altitude of 600 m, as well as parched woods in India. This medium-sized tree grows to a height of 40 m. Tree’s rough bark is corky grey to grey-white coloured with few tessellations. Outer bark is sliced, a lot of milky latex that oozes out. Both the branches and the leaves are spirally arranged, meaning that several leaves emerge from a single point. The tree has a lovely shape due to orderly branching and whorls of 4–7 dark green, leathery, slightly rounded leaves. *Alstonia scholaris* tree bark also known as ‘Dita’ used as a folk medicine to treat fever, diarrhoea, and other symptoms. Tribal people from Western Ghats had little knowledge about the tree and afraid of the devil’s tree and avoid to sit under or near this tree.

Traditionally, *Alstonia* Plant comprised of medicinal properties and have been utilized for preparation of bitter tonic, aphrodisiac, febrifuge, stimulant, expectorant, carminative, astringent, and stomachic properties from ancient times (Singh & Panda, 2005). The milky

juice of this tree contains curative properties to cure ulcers, wounds, and rheumatic aches and it relieves ear pain on combining with oil. The *Alstonia* bark possesses the curative properties and is used to cure lung associated disorders like asthma, lung cancer, pneumonia and also hypertension. While leaf extracts are used as febrifuge, the bark tincture and leaf juice can both function as potent galactagogues (Channa *et al.*, 2005). Ayurveda describes the *Alstonia* plant as a bitter and astringent herb for treating skin disorders, malarial fever, chronic dysentery, diarrhoea, urticaria, snake bites and in the purification process of *Panchakarma* (body cleanse).

2. MATERIALS AND METHODS

A. Collection and processing of plant material

The plant *Alstonia scholaris* was collected from Gwalior district (M.P.), India. The plant leaves were washed under running tap water, air dried in the shade and ground to a fine powder and stored in the airtight bottle.

B. Preparation of Leaf extract

Leaf powder (10 g) of the *Alstonia scholaris* was extracted in 100 ml methanol and double distilled water separately using the Soxhlet apparatus. The filtrates were concentrated on a rotary evaporator and then stored at 4°C till further use (Swaminathan, *et al.*, 2017).

C. Phytochemicals screening

The qualitative phytochemicals screening of crude leaf extract was carried out using standard phytochemical methods with slight modifications (Harborne, 1998; Evans, 1989; Santhi & Swaminathan, 2011).^[18,19 and 20] The positive tests were noted as weak (+), moderate (++), strong (+++) and absent (-).

D. Synthesis of Metal complex or Nanoparticle Using Green method

The synthesis of Selenium nanoparticles (SeNPs) using *Alstonia scholaris* was done using the modified method of Alagesan and Venugopal, (2019).

E. Determination of antimicrobial activity

i) Bacterial strains

Klebsiella pneumonia (MTCC 39), *Salmonella enterica* (MTCC 098), *Candida albicans* (MTCC 227) and *E.coli* (MTCC 1687) used in the present study were procured from the MTCC, Chandigarh, India.

ii) Antimicrobial activity

The antimicrobial activities of *Alstonia scholaris* leaves extracts were determined using the agar-well diffusion method following a procedure with slight modification. The test bacterial strain was inoculated in to nutrient broth and incubated at 37°C for 24 hours. The cultures were later diluted with sterile nutrient broth, adjusted to 0.5 McFarland turbidity standards and inoculated on Mueller-Hinton agar plates by spreading the culture over the surface of the medium using a sterile cotton swab. Wells of 6 mm diameter were punched in the medium using a sterile cork borer and filled with 100 µL of crude leaves extracts in different concentrations 25 µg/µl, 50 µg/µl, 75µg/µl and 100µg/µl dissolved in solvent dimethyl sulfoxide (DMSO). Positive controls were also run in parallel on the same plate with di methyl sulfoxide and streptomycin and Fluconazole (25µg/µl), respectively. The plates were incubated in an upright position at 37°C for overnight in an incubator. At the end of incubation period, antibacterial activities were assessed by measuring the diameters of the zone of inhibition around each well, excluding the diameter of well in mm.^[21]

3. RESULT AND DISCUSSION

Present study was carried out on plant leaves of *Alstonia scholaris* collected from Botany department premise of Gwalior district (MP) India. Present study is carried out assess the presence phytochemicals and evaluation of antimicrobial activity against bacteria and fungi procured from MTCC, Chandigarh.

3.1 Phytochemical screening of Methanolic and Aqueous extract from *Alstonia scholaris*

In the present study, aqueous and methanol extracts were assessed for phytochemical screening and found the presence of alkaloids, flavonoids, phlobatannins, phenolic, saponins and tannins in methanol extract. While alkaloids, flavonoids, saponins, tannins and phenolics were present in aqueous extract as shown in table 1.

Ornamental plants also comprised of medicinal properties, can be serve as therapeutic agents as needs. The bioactive constituentsincorporates molecules vital for normal cellular metabolisms, offering external sources to sustain cellular activities, especially those crucial in various cellular disorders. This study stated the presence of biologically significant phytochemicals, including alkaloids, flavonoids, glycosides, saponins, tannins, and phenolic present in the aqueous and methanol extract of *Alstonia scholaris* leaves. Study of Khyade and Vaikos (2009) supported the present work. Khyade and Vaikos (2009) also observed the presence of alkaloids, flavonoids, phlobatannins, phenolic, saponins and tannins in methanol

extract. Misra *et al.*, (2011) also find the same results when studies the methanol and aqueous extracts of *Alstonia scholaris*.

Table 3.1. Phytochemical components of methanol and aqueous solvent leaves extracts of *A. scholaris*.

Phytochemical constituents	Aqueous	Methanol
Alkaloids	+++	+++
Anthraquinone	--	--
Cardiac glycoside	--	--
Flavonoids	++	++
Phlobatannin	--	++
phenolics	++	+++
Steroids	--	--
Saponins	++	+++
Tannins	++	+++
Terpenoid	--	--

Misra *et al.*, (2011) observed the presence of alkaloids, terpenoids, saponins and steroids, but in the present study steroids was found absent in aqueous extract.

3.2 Antimicrobial study

In the present study, AS-SeM NP **and** AS-SeAq NP from leaf extracted in methanol and aqueous solvent were used. Methanol, aqueous extracts and concerned AS-SeM NP and AS-SeAq NP were used in phytochemical screening as well as antimicrobial activity. Antimicrobial study was carried out to detect the efficacy of present bioactive molecules in both extracts, against bacteria and fungi. Antimicrobial activity was carried out against the bacteria- *E.coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, and fungi- *Candida albicans*. Significant variation was observed for antimicrobial activity against pathogens using both extracts. Both extracts were used in a range of concentration; 25, 50, 75 and 100 µg/µl. While the standard streptomycin was used in a concentration of 25 µg/µl.

Table 3.2.: Antimicrobial activity of AS-SeM NP.

AS-SeM NP from <i>Alstonia scholaris</i> methanolic Extract (Zone of Inhibition in mm)				
Conc. in (µg/µl)	<i>E.coli</i>	<i>Salmonella</i>	<i>Klebsiella</i>	<i>C.albicans</i>
25	13.3±1.3	10.9.0±0.2	15.0±1.3	11.0±0.7
50	15.8±0.8	13.0±0.5	18.0±1.7	13.3±1.1
75	19.9±0.8	16.8±1.8	22.6±1.4	15.6±0.2
100	20.8±1.2	19.2±0.6	21.5±1.3	17.4±0.3
Streptomycin (25µg/µl)	21.0±0.4	20.5±1.1	23.3±1.2	0
Fluconazole (25µg/µl)	0	0	0	18.8±0.2

*Each presented value in table are expressed as mean $\pm$ SD Means of triplicate analyses (n=3) AS-SeM: *Alstonia scholaris* Selenium nanoparticles (NP) synthesized from Methanolic (M) extract. 11111111.

AS-SeM NP gave significant activity against *E.coli*, *Klebsiella pneumoniae* and *candida albicans*. Whereas aqueous extract was found effective against *salmonella enterica*.

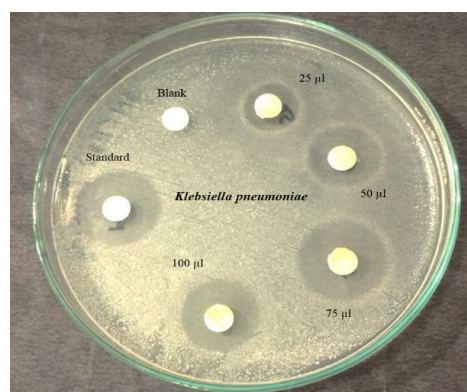
Inhibition zone ranged from 13.3 ± 1.3 mm to 20.8 ± 1.2 against *E.coli*, 15.0 ± 1.3 to 23.3 ± 1.2 mm and 11.0 ± 0.7 to 17.4 ± 0.3 mm against *candida albicans*. Swamy *et al.*, (2019) also find the similar results for methanol extract against *Candida albicans*, the inhibitions zones were 11mm and 14 mm at 50 $\mu\text{g}/\mu\text{l}$ and 100 $\mu\text{g}/\mu\text{l}$ respectively. However Swamy *et al.*, observed the inhibition zone of 15 mm for both concentration 50 $\mu\text{g}/\mu\text{l}$ & 100 $\mu\text{g}/\mu\text{l}$ against *E.coli*. Study of Swamy *et al.*, (2019) supports the present antimicrobial activity against the *E.coli* and *Candida albicans*.

Table 3.3. Antimicrobial activity of AS-SeAq NP.

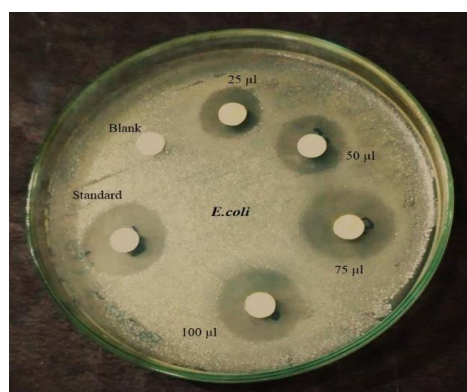
AS-SeAq NP from <i>Alstonia scholaris</i> aqueous Extract (Zone of Inhibition in mm)				
Conc. in ($\mu\text{g}/\mu\text{l}$)	<i>E.coli</i>	<i>Salmonella</i>	<i>Klebsiella</i>	<i>C.albicans</i>
25	11.3 ± 1.6	$11.5.0 \pm 1.2$	12.0 ± 0.25	10.8 ± 1.4
50	13.2 ± 1.1	12.6 ± 1.5	13.1 ± 1.3	11.9 ± 0.9
75	14.0 ± 0.7	14.1 ± 1.3	15.0 ± 1.1	14.2 ± 1.3
100	16.1 ± 0.5	17.2 ± 1.2	16.0 ± 0.5	16.5 ± 1.1
Streptomycin (25 $\mu\text{g}/\mu\text{l}$)	20.9 ± 0.2	20.5 ± 1.1	21.5 ± 1.3	0
Fluconazole (25 $\mu\text{g}/\mu\text{l}$)	0	0	0	18.9 ± 0.5

*Each presented value in table are expressed as mean $\pm$ SD Means of triplicate analyses (n=3)

AS-SeAq: *Alstonia scholaris* Selenium nanoparticles (NP) synthesized from aqueous (Aq) extract.



(A)



(B)

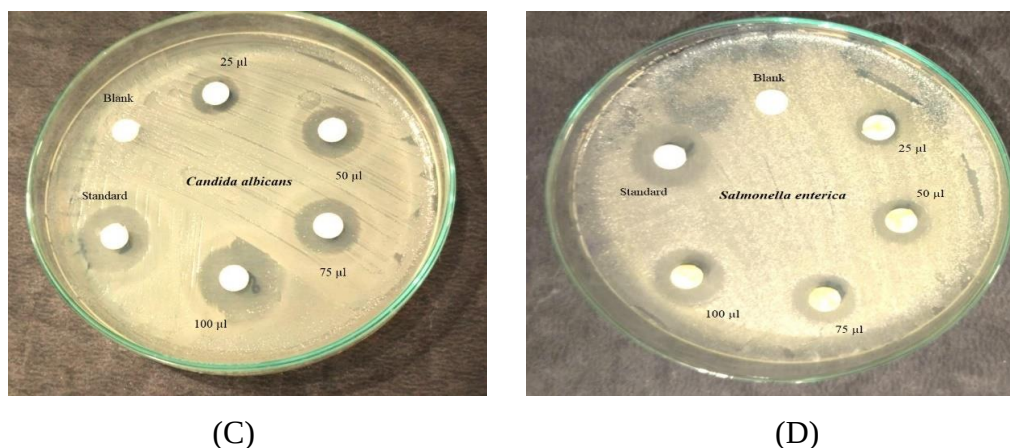


Figure 3.1: Antimicrobial activity of methanolic extract of *Alstonia scholaris*'s leaves against *Klebsiella pneumonia* (A), *E.coli* (B) and *Candida albicans* (C); antimicrobial activity of aqueous extract of *Alstonia scholaris*'s leaves against *Salmonella enterica* (D)

4. CONCLUSION

In the present study, methanol and aqueous extraction of *Alstonia scholaris* leaves concluded that the extraction in methanol could have been produced more active bioactive compounds responsible for many biological activities comparatively to aqueous extraction. The extensive investigation is continued to find the more significant bioactive compounds from the *Alstonia scholaris* leaves for ethno botanical and alternative medicinal use for various ailments. Previous studies also states the presence of anticancerous, antimicrobial, molluscicidal, anxiolytic and antipsychotic agent in the *Alstonia scholaris*.

Therefore, this study is recommended *Alstonia scholaris* as a plant of therapeutically importance, the more spectral analysis must be required to evaluate the mechanism of action of significant compounds isolated from *Alstonia scholaris* for their medicinal significance with fewer side effects as futuristic safe drugs.

5. ACKNOWLEDGEMENT

Author is wished to acknowledge **Advanced Environmental Testing and Research lab (P) Ltd, Gwalior (M.P.) 474011** facilities to carry out this work.

REFERENCES

1. Alagesan V, Venugopal S. 2019. Green synthesis of selenium nanoparticle using leaves extract of *Withania somnifera* and its biological applications and photocatalytic activities. *Bionanoscience*, 9(1): 105-116.

2. Chandra Shekhar Misra, Kumar Pratyush, Lipin Dev M.S, Joel James, Arun Kumar Thaliyil Veettil and Thankamani V A comparative study on phytochemical screening and antibacterial activity of roots of *Alstonia scholaris* with the roots, leaves and stem bark, *Int. J. Res. Phytochem. Pharmacol.*, 2011; 1(2): 77-82.
3. Channa, S., Dar, A., Ahmed, S., & Atta-Ur-Rahman. Evaluation of *Alstonia scholaris* leaves for broncho-vasodilatory activity. *Journal of Ethnopharmacology*, 2005; 97(3): 469–476. <https://doi.org/10.1016/j.jep.2004.12.009>
4. Chhajed, M., Jain, A., Pagariya, A., Dwivedi, S., Jain, N., & Taile, V. *Alstonia scholaris* Linn. R. Br.: An assessment of its botany, conventional utilization, phytochemistry and pharmacology. *Pharmacognosy Reviews*, 2023; 184–203. <https://doi.org/10.5530/097627870302>
5. Eloff JN. On expressing the antibacterial activity of plant extracts a small first step in applying scientific knowledge to rural primary health care. *South African Journal of Science*, 2000; 96: 116-8.
6. Khyade, M. S.* and Vaikos, N. P. Phytochemical and antibacterial properties of leaves of *Alstonia scholaris* R. Br. *African Journal of Biotechnology*, 2009; 8(22): 6434-6436.
7. Martini N, Eloff JN. The preliminary isolation of several anti-bacterial compounds from *Combretum erythrophyllum* (Combretaceae). *Journal of ethnopharmacology*. 1998; 62(3): 255-63.
8. Mukherjee PK, Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines, *J Ethnopharmacol*, 2006; 103: 2535.
9. Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. *Lancet* (London, England). 1997; 349(9061): 1269-76.
10. Peterson LR, Dalhoff A. Towards targeted prescribing: will the cure for antimicrobial resistance be specific, directed therapy through improved diagnostic testing? *The Journal of antimicrobial chemotherapy*, 2004; 53(6): 902-5.
11. Phukan Parmita, Phukan SN. Phytochemical and Pharmacognostic Analysis of *Alstonia scholaris* (L) R. BR., A commonly available Medicinal Plant in Assam, India, *Research Journal of Chemical Sciences*, 2014; 4(11): 68-71.
12. Singh, M. P., & Panda, H. (2005). *Medicinal herbs with their formulations*. Daya Books.
13. Tamilarasi T, Ananthi T. Phytochemical Analysis and Anti-Microbial Activity of *Mimosa pudica* Linn., *Res. J. chem. sci.*, 2012; 2(2): 72-74.