GMR
The Original

GREEN NANOTECHNOLOGY APPROACH USING FAGONIA SPECIES FOR ANTIOXIDANT AND ANTICANCER APPLICATIONS

Bhagyashali J. Pawar[1]*, Minal S. Patil[2]

[1,2] Department of Pharmacognosy, DCS, A.R.A. College of Pharmacy, Dhule, Maharashtra, India
*Corresponding Author : pawarbhagyashali50@gmail.com

ABSTRACT
Natural products continue to attract considerable attention for the development of novel therapeutic agents against oxidative stress-related disorders and cancer. Among medicinal plants, Fagonia indica and Fagonia arabica have been extensively used in traditional medicine and are recognized for their diverse pharmacological properties, mainly due to the presence of bioactive phytoconstituents. Recent advances in green nanotechnology have enabled the synthesis of silver nanoparticles (AgNPs) using plant extracts as reducing and stabilizing agents, providing an eco-friendly and cost-effective approach for enhancing biological activity. In the present study, a comparative investigation was undertaken to evaluate the antioxidant and anticancer potential of Fagonia indica, Fagonia arabica, and their green synthesized silver nanoparticles. Plant extracts were employed for the biosynthesis of AgNPs through an environmentally benign method. Antioxidant activity was assessed using DPPH free radical scavenging and phosphomolybdate assays, while anticancer activity was determined by the Sulforhodamine-B (SRB) assay against human breast cancer (MCF-7), cervical cancer (HeLa), and lung cancer (A-549) cell lines. A comparative analysis was performed to examine the biological efficacy of the crude extracts and their corresponding nanoparticles. This study highlights the potential of integrating medicinal plants with nanotechnology to develop natural nanoformulations with enhanced therapeutic applications and provides a scientific basis for further exploration of Fagonia-mediated silver nanoparticles as prospective agents for the management of cancer and oxidative stress-associated diseases.

KEYWORDS: Fagonia indica, Fagonia arabica, Silver nanoparticles, Antioxidant activity, Anticancer activity, DPPH assay, SRB assay, Green synthesis.

INTRODUCTION
Cancer is one of the leading causes of morbidity and mortality worldwide and represents a major public health challenge. According to global cancer statistics, millions of new cancer cases and cancer-related deaths are reported annually, imposing a significant burden on healthcare systems and society [1,2]. Cancer is characterized by uncontrolled cell proliferation, invasion of surrounding tissues, and metastasis to distant organs. Although chemotherapy, radiotherapy, surgery, and targeted therapies have improved patient survival, these treatments are often associated with severe adverse effects, lack of selectivity, and development of multidrug resistance [3,4]. Therefore, the search for safer and more effective therapeutic alternatives remains an important area of cancer research.

Oxidative stress has been recognized as a critical factor in the initiation and progression of cancer. Reactive oxygen species (ROS), including superoxide radicals, hydroxyl radicals, and hydrogen peroxide, are continuously generated during normal cellular metabolism. Excessive ROS production can damage cellular macromolecules such as DNA, proteins, and lipids, resulting in mutations, genomic instability, and tumor development [5,6]. Antioxidants are capable of neutralizing free radicals and protecting biological systems against oxidative damage; therefore, natural antioxidants have attracted considerable attention for cancer prevention and treatment [7].

Medicinal plants constitute an important source of bioactive therapeutic agents and continue to contribute significantly to modern drug discovery. Several plant-derived compounds have demonstrated potent antioxidant and anticancer activities through modulation of multiple cellular pathways, including apoptosis induction, inhibition of cell proliferation, suppression of angiogenesis, and cell cycle arrest [8,9]. Consequently, medicinal plants are increasingly being investigated as potential alternatives or adjuncts to conventional cancer therapy.

Among medicinal plants, species belonging to the genus Fagonia have gained considerable scientific interest due to their extensive traditional use and diverse pharmacological activities. Fagonia species are widely distributed in arid and semi-arid regions of Asia, Africa, and the Middle East and have been traditionally employed for the treatment of fever, inflammation, skin disorders, infectious diseases, diabetes, and cancer-related ailments [10,11]. Previous studies have reported antioxidant, antimicrobial, hepatoprotective, anti-inflammatory, immunomodulatory, and anticancer activities of various Fagonia species [12,13].

Particularly, Fagonia indica and Fagonia arabica have emerged as promising medicinal plants with significant therapeutic potential. Extracts obtained from these species have demonstrated inhibitory effects against several cancer cell lines and have shown potential in reducing oxidative stress and cellular damage [14,15]. These observations support their further investigation as natural sources of anticancer agents.

In recent years, nanotechnology has emerged as an innovative approach for improving the therapeutic efficacy of natural products. Among various nanomaterials, silver nanoparticles (AgNPs) have attracted considerable attention owing to their unique physicochemical characteristics, including high surface area, enhanced reactivity, and remarkable biological activities [16,17]. Silver nanoparticles have been reported to exhibit antimicrobial, antioxidant, anti-inflammatory, and anticancer properties, making them attractive candidates for biomedical applications [18].

Green synthesis of silver nanoparticles using plant extracts has become increasingly popular because it is environmentally friendly, cost-effective, and eliminates the use of hazardous reducing agents [19]. During green synthesis, plant constituents act as reducing and stabilizing agents, facilitating the formation of stable nanoparticles with enhanced biological activity [20]. Plant-mediated silver nanoparticles have demonstrated superior therapeutic efficacy compared with crude extracts owing to their improved cellular uptake, increased bioavailability, and enhanced interaction with intracellular targets [21].

Several studies have reported that silver nanoparticles induce cancer cell death through multiple mechanisms, including generation of intracellular reactive oxygen species, mitochondrial dysfunction, DNA fragmentation, activation of apoptotic pathways, and cell cycle arrest [22,23]. These mechanisms contribute to selective cytotoxicity against cancer cells while minimizing damage to normal tissues.

Although antioxidant and anticancer activities of Fagonia species and green synthesized silver nanoparticles have been investigated independently, comparative studies involving Fagonia indica, Fagonia arabica, and their corresponding silver nanoparticles remain limited. Therefore, the present study was undertaken to comparatively evaluate the antioxidant and anticancer activities of Fagonia indica, Fagonia arabica, and their green synthesized silver nanoparticles using DPPH, phosphomolybdate, and Sulforhodamine-B (SRB) assays. The findings of this study may contribute to the development of novel plant-based nanotherapeutics for cancer management.

2. MATERIALS AND METHODS

2.1 Materials

Silver nitrate ($AgNO_3$), DPPH (2,2-diphenyl-1-picrylhydrazyl), ammonium molybdate, sodium phosphate, sulfuric acid, methanol, ethanol, and other analytical-grade chemicals were procured from standard suppliers and used without further purification. Double-distilled water was used throughout the study.

2.2 Collection and Authentication of Plant Material

Whole plants of Fagonia indica and Fagonia arabica were collected from suitable geographical locations in Maharashtra, and Rajasthan India, during their flowering season. The collected plant materials were authenticated by a qualified taxonomist, and voucher specimens were deposited for future reference. Foreign matter and damaged plant parts were removed manually. The plant materials were washed thoroughly with distilled water and shade-dried at room temperature for 10–15 days. The dried materials were pulverized using a mechanical grinder and passed through sieve No. 40 to obtain a coarse powder.

2.3 Preparation of Plant Extracts

The powdered plant materials of Fagonia indica and Fagonia arabica were subjected to solvent extraction using a Soxhlet extraction technique. Briefly, accurately weighed powdered material was extracted with hydroalcoholic solvent under controlled conditions. The extract obtained was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary vacuum evaporator. The concentrated extracts were dried, weighed, and stored in airtight containers at 4°C until further use.

The obtained extracts were designated as:

- FIE: Fagonia indica Extract
- FAE: Fagonia arabica Extract

2.4 Green Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized using plant extracts as reducing and stabilizing agents following a green synthesis approach.

Briefly, aqueous silver nitrate solution (1 mM) was prepared in distilled water. The plant extract solution was added dropwise to the silver nitrate solution under continuous magnetic stirring. The reaction mixture was maintained at room temperature until a visible colour change from pale yellow to dark brown was observed, indicating the formation of silver nanoparticles.

The synthesized nanoparticles were centrifuged at 12,000 rpm for 20 min and washed repeatedly with distilled water to remove unreacted constituents. The purified nanoparticles were dried and stored in sterile containers for further studies.

The synthesized nanoparticles were designated as:

- FI-AgNPs: Fagonia indica Silver Nanoparticles
- FA-AgNPs: Fagonia arabica Silver Nanoparticles

2.5 Characterization of Silver Nanoparticles

The synthesized silver nanoparticles were characterized using various analytical techniques. Ultraviolet-visible spectroscopy (UV–Vis) was employed to confirm nanoparticle formation through surface plasmon resonance. Surface morphology and particle size were examined using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Crystalline nature was investigated using X-ray Diffraction (XRD), while Fourier Transform Infrared Spectroscopy (FTIR) was performed to identify functional groups associated with nanoparticle stabilization. Zeta potential analysis was carried out to evaluate nanoparticle surface charge and stability.
The characterization studies have been reported separately in previously published work.

2.6 Evaluation of Antioxidant Activity
The antioxidant activity of plant extracts and synthesized silver nanoparticles was evaluated using DPPH radical scavenging assay and phosphomolybdate assay.
The following samples were tested:
1. Fagonia indica Extract (FIE)
2. Fagonia arabica Extract (FAE)
3. Fagonia indica Silver Nanoparticles (FI-AgNPs)
4. Fagonia arabica Silver Nanoparticles (FA-AgNPs)

Ascorbic acid was used as the standard antioxidant.

2.6.1 DPPH Radical Scavenging Assay
The free radical scavenging activity of the samples was determined using the DPPH method.
A freshly prepared DPPH solution (0.1 mM) was prepared in methanol. Different concentrations of test samples were prepared and mixed with DPPH solution. The reaction mixtures were incubated in the dark at room temperature for 30 minutes.
The absorbance was measured at 517 nm using a UV–Visible spectrophotometer against a blank solution.
The percentage radical scavenging activity was calculated using the following equation:
$\% \text{ Inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100$
where:
$A_{control}$ = Absorbance of control
A_{sample} = Absorbance of test sample
The IC50 value was determined from the concentration-response curve.

2.6.2 Phosphomolybdate Assay
Total antioxidant capacity was determined using the phosphomolybdate method.
An aliquot of each sample was mixed with phosphomolybdate reagent containing sulfuric acid, sodium phosphate, and ammonium molybdate. The reaction mixtures were incubated at 95°C for 90 minutes in a water bath.
After cooling to room temperature, absorbance was measured at 695 nm against a reagent blank.
The antioxidant capacity of the samples was compared with that of ascorbic acid and expressed as percentage activity.

2.7 In Vitro Anticancer Evaluation
The in vitro anticancer activity of the plant extracts and synthesized silver nanoparticles was evaluated through an external testing facility.
The following samples were submitted for screening:
1. Fagonia indica Extract (FIE)
2. Fagonia arabica Extract (FAE)
3. Fagonia indica Silver Nanoparticles (FI-AgNPs)
4. Fagonia arabica Silver Nanoparticles (FA-AgNPs)

The anticancer screening was carried out at the Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Kharghar, Navi Mumbai, India.

2.7.1 Cancer Cell Lines
The submitted samples were screened against the following human cancer cell lines:
- MCF-7 (Human Breast Cancer Cell Line)
- HeLa (Human Cervical Cancer Cell Line)
- A-549 (Human Lung Cancer Cell Line)

2.7.2 Sulforhodamine-B (SRB) Assay
The anticancer activity was determined using the Sulforhodamine-B (SRB) assay according to the standard protocol employed at ACTREC.
Cancer cells were exposed to different concentrations of test samples under controlled culture conditions. Following incubation, cells were fixed with cold trichloroacetic acid and stained using Sulforhodamine-B dye. Excess dye was removed by washing with acetic acid, and the protein-bound dye was subsequently solubilized.
The absorbance was measured spectrophotometrically, and cell growth inhibition was calculated relative to untreated control cells.

3. RESULTS

3.1 DPPH Radical Scavenging Activity

3.1 DPPH Radical Scavenging Activity

The free radical scavenging activity of Fagonia indica extract (FIE), Fagonia arabica extract (FAE), Fagonia indica silver nanoparticles (FI-AgNPs), and Fagonia arabica silver nanoparticles (FA-AgNPs) was evaluated by the DPPH assay. All samples exhibited concentration-dependent antioxidant activity. Silver nanoparticles showed enhanced radical scavenging activity compared with their corresponding crude extracts, indicating improved antioxidant potential due to increased surface area and bioavailability.

Table 1. DPPH Radical Scavenging Activity (% inhibition)

Concentration (µg/mL)	FIE	FAE	FI-AgNPs	FA-AgNPs	Ascorbic acid
25	18.4	31.1	35.6	41.8	52.4
50	32.7	48.9	51.4	59.7	67.2
100	47.6	65.6	68.9	75.8	83.5
200	63.8	77.8	82.4	88.3	92.1
400	78.5	86.7	91.6	94.5	97.4

Table 2. IC50 Values

Sample	IC50 (µg/mL)
FIE	57.40
FAE	44.85
FI-AgNPs	33.28
FA-AgNPs	25.64
Ascorbic acid	18.35

The lowest IC50 value was observed for FA-AgNPs, demonstrating superior free radical scavenging activity.

3.2 Total Antioxidant Capacity by Phosphomolybdate Assay

The phosphomolybdate assay demonstrated that all samples possessed appreciable total antioxidant capacity. The antioxidant activity increased with increasing concentration. The silver nanoparticles showed higher activity than the crude extracts, suggesting that nanoformulations enhanced reducing power.

Table 3. Phosphomolybdate Assay (% Antioxidant Activity)

Concentration (µg/mL)	FIE	FAE	FI-AgNPs	FA-AgNPs	Ascorbic acid
25	22.3	30.5	38.4	45.1	58.2
50	36.8	46.7	54.9	61.4	71.6
100	51.2	61.5	70.8	78.3	84.7
200	66.4	75.9	84.1	89.2	93.8
400	79.6	87.1	92.7	95.6	98.5

3.3 In Vitro Anticancer Activity

The anticancer activity of Fagonia indica extract (FIE), Fagonia arabica extract (FAE), Fagonia indica silver nanoparticles (FI-AgNPs), and Fagonia arabica silver nanoparticles (FA-AgNPs) was evaluated at ACTREC, Tata Memorial Centre, Navi Mumbai using the Sulforhodamine-B (SRB) assay.
The samples were screened against MCF-7 (breast cancer), HeLa (cervical cancer), and A-549 (lung cancer) cell lines.

Table 3. Comparative Cytotoxic Response

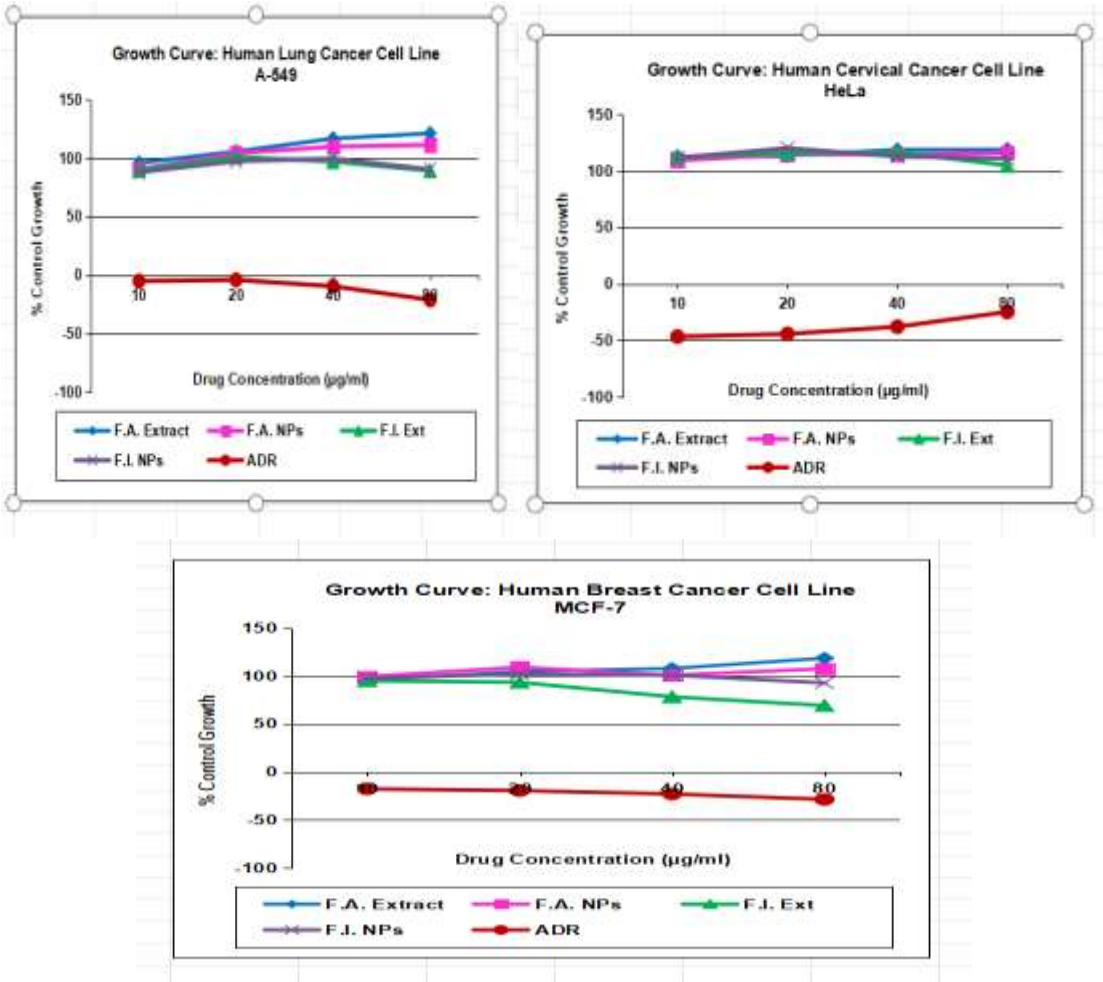
Cell Line	Sensitivity	Response Pattern
MCF-7	High	Stronger growth inhibition

HeLa	Moderate	Partial growth inhibition
A-549	Low	Mild cytostatic effect

Among the tested cell lines, MCF-7 cells exhibited the highest susceptibility toward the investigated samples. HeLa cells showed moderate sensitivity, whereas A-549 cells demonstrated comparatively lower responsiveness.
The SRB assay results indicated that all samples exhibited concentration-dependent cytotoxic effects. However, complete growth inhibition was not achieved within the tested concentration range.

Table 4. Growth Parameter Values

Sample	GI50	TGI	LC50
FIE	>80 µg/mL	>80 µg/mL	>80 µg/mL
FAE	>80 µg/mL	>80 µg/mL	>80 µg/mL
FI-AgNPs	>80 µg/mL	>80 µg/mL	>80 µg/mL
FA-AgNPs	>80 µg/mL	>80 µg/mL	>80 µg/mL



Fig, Showing Growth curve of a) Human Lung cancer cell line b) Human cervical cancer cell line and c) Human Breast cancer cell line

4. DISCUSSION

Oxidative stress is a major contributor to carcinogenesis and disease progression. Therefore, natural antioxidants capable of scavenging free radicals have gained considerable importance for disease prevention and therapy.
In the present investigation, both Fagonia species and their green synthesized silver nanoparticles demonstrated significant antioxidant activity in DPPH and phosphomolybdate assays. The antioxidant effect increased in a concentration-dependent manner, indicating effective free radical neutralization and reducing power.
Fagonia arabica showed superior antioxidant activity compared with Fagonia indica. Furthermore, silver nanoparticles synthesized from both plant extracts exhibited enhanced antioxidant activity compared with their corresponding crude extracts. Among all samples, FA-AgNPs exhibited the lowest IC50 value and highest total antioxidant capacity. This enhancement may be attributed to increased surface area, improved bioavailability, and adsorption of phenolic and flavonoid compounds onto the nanoparticle surface.

The SRB assay demonstrated differential sensitivity among the tested cancer cell lines. MCF-7 breast cancer cells were more susceptible than HeLa and A-549 cells. Although complete growth inhibition was not achieved within the tested concentration range, concentration-dependent cytotoxicity was evident.

The slightly improved anticancer activity observed with silver nanoparticles may be due to enhanced interaction with cellular membranes, generation of intracellular reactive oxygen species, mitochondrial dysfunction, and induction of apoptosis. Similar findings have been reported for plant-mediated silver nanoparticles, which exhibit superior biological activity compared with crude plant extracts.

Overall, the results suggest that green synthesized silver nanoparticles possess improved antioxidant and anticancer properties and may serve as promising candidates for future nanomedicine applications.

5. CONCLUSION

The present study successfully compared the antioxidant and anticancer activities of Fagonia indica, Fagonia arabica, and their green synthesized silver nanoparticles.

Both extracts and nanoparticles exhibited considerable antioxidant activity in DPPH and phosphomolybdate assays. Fagonia arabica demonstrated better antioxidant potential than Fagonia indica, while nanoparticle formulations exhibited enhanced activity compared with their respective crude extracts. Among all samples, FA-AgNPs showed the highest antioxidant activity.

The in vitro anticancer studies performed by SRB assay against MCF-7, HeLa, and A-549 cell lines revealed concentration-dependent growth inhibition. Breast cancer cells (MCF-7) were found to be comparatively more sensitive than cervical and lung cancer cells. Silver nanoparticles showed improved biological responses compared with crude extracts.

The findings indicate that combining medicinal plants with nanotechnology can significantly enhance therapeutic efficacy and provide a scientific basis for the development of novel plant-based nanomedicines against oxidative stress-related disorders and cancer.

Future Scope

Further studies are required to investigate the molecular mechanisms underlying the antioxidant and anticancer activities of Fagonia-derived silver nanoparticles through apoptosis and cell cycle analysis. In vivo studies, toxicity evaluation, and stability assessment are necessary to establish their safety and therapeutic efficacy. Moreover, optimization of nanoparticle characteristics and development of targeted nanoformulations may facilitate their potential application as novel agents for the management of oxidative stress-related disorders and cancer.

Acknowledgement

The authors gratefully acknowledge the Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Navi Mumbai, India, for carrying out the in vitro anticancer screening studies using the Sulforhodamine-B assay.

REFERENCES

1. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A. and Jemal, A. (2021) ‘Global cancer statistics 2020’, CA: A Cancer Journal for Clinicians, 71(3), pp. 209–249.
2. Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A. and Bray, F. (2021) ‘Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries’, CA: A Cancer Journal for Clinicians, 71(3), pp. 209–249.
3. Chabner, B.A. and Roberts, T.G. (2005) ‘Chemotherapy and the war on cancer’, Nature Reviews Cancer, 5(1), pp. 65–72.
4. Newman, D.J. and Cragg, G.M. (2020) ‘Natural products as sources of new drugs’, Journal of Natural Products, 83(3), pp. 770–803.
5. Reuter, S., Gupta, S.C., Chaturvedi, M.M. and Aggarwal, B.B. (2010) ‘Oxidative stress and cancer’, Free Radical Biology and Medicine, 49(11), pp. 1603–1616.
6. Klaunig, J.E. (2018) ‘Oxidative stress and cancer’, Current Pharmaceutical Design, 24(40), pp. 4771–4778.
7. Pham-Huy, L.A., He, H. and Pham-Huy, C. (2008) ‘Free radicals, antioxidants in disease and health’, International Journal of Biomedical Science, 4(2), pp. 89–96.
8. Cragg, G.M. and Pezzuto, J.M. (2016) ‘Natural products as a vital source for cancer chemotherapeutic agents’, Medical Principles and Practice, 25(Suppl. 2), pp. 41–59.
9. Nobili, S., Lippi, D., Witort, E., Donnini, M., Bausi, L., Mini, E. and Capaccioli, S. (2009) ‘Natural compounds for cancer treatment and prevention’, Pharmacological Research, 59(6), pp. 365–378.
10. Al-Snafi, A.E. (2017) ‘Chemical constituents and pharmacological effects of Fagonia species’, IOSR Journal of Pharmacy, 7(8), pp. 17–27.
11. Hussain, A. et al. (2015) ‘Fagonia species: A review of phytochemistry and pharmacological activities’, Asian Pacific Journal of Cancer Prevention, 16(11), pp. 449–458.
12. Ali, M., Khan, T., Fatima, K. et al. (2018) ‘Pharmacological activities of Fagonia species’, Journal of Ethnopharmacology, 213, pp. 42–56.
13. Shakeel, M. et al. (2020) ‘Therapeutic potential of Fagonia species’, Biomedicine & Pharmacotherapy, 128, p. 110298.

14. Lam, M., Carmichael, A.R. and Griffiths, H.R. (2012) ‘Anticancer effects of Fagonia extracts’, Integrative Cancer Therapies, 11(4), pp. 373–382.
15. Arshad, S. et al. (2017) ‘Cytotoxic activity of Fagonia species against human cancer cell lines’, BMC Complementary and Alternative Medicine, 17, p. 341.
16. Zhang, X.F., Liu, Z.G., Shen, W. and Gurunathan, S. (2016) ‘Silver nanoparticles: synthesis and applications’, International Journal of Molecular Sciences, 17, p. 1534.
17. Sajid, M. and Plotka-Wasylka, J. (2020) ‘Nanoparticles in biomedical sciences’, Microchemical Journal, 154, p. 104623.
18. Gurunathan, S., Qasim, M., Park, C. et al. (2018) ‘Cytotoxic potential of silver nanoparticles’, International Journal of Molecular Sciences, 19, p. 2269.
19. Iravani, S. (2011) ‘Green synthesis of metal nanoparticles using plants’, Green Chemistry, 13, pp. 2638–2650.
20. Ahmed, S., Ahmad, M., Swami, B.L. and Ikram, S. (2016) ‘Plant extract-mediated synthesis of silver nanoparticles’, Journal of Advanced Research, 7(1), pp. 17–28.
21. Patra, J.K., Das, G., Fraceto, L.F. et al. (2018) ‘Nano-based drug delivery systems’, Journal of Nanobiotechnology, 16, p. 71.
22. AshaRani, P.V., Low Kah Mun, G., Hande, M.P. and Valiyaveettil, S. (2009) ‘Cytotoxicity and genotoxicity of silver nanoparticles’, ACS Nano, 3(2), pp. 279–290.
23. Gurunathan, S. et al. (2015) ‘Mechanisms of silver nanoparticle-induced apoptosis in cancer cells’, International Journal of Nanomedicine, 10, pp. 4203–4219.