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Pharmacological Significance of green synthesis of Nanoparticles from medicinal plant Withania somnifera

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ABSTRACT :

In present scenario, the increased use of chemically synthesized medications lead oxidation and stress- causing chronic diseases diabetes, cancer, BP problems, neurological disorders, Alzheimer disease and poor immunity. On the large scale usage of antibiotics are also detrimental to health due to developing of multidrug resistant bacteria against the high dose antibiotics in human. Regardless of advances in synthetic drug development, natural medicinal compounds from plants are emerging as new natural medications to cure incurable diseases or the side effects of synthetic drugs. Presently tedious works are carrying out to develop new techniques for the isolation; identification and characterization of new natural compounds from plant sources. Nanotechnology is being used extensively in Green synthesis of nanoparticals. Extensive researches are still going on in the search of new natural compounds with least side effects. Withania somnifera also known as Aswagandha is a medicinal plant having wonderful medicinal properties - antioxidant and antimicrobial activities. Withania somnifera possess flavonids, alkaloids, phenolic compounds, steroids, tannins, cardiac glycosides. This makes the plant as a significant source of green synthesis of nanoprticles using gold, silver copper, zinc, selenium and so on. The present review insights the current status of green synthesis of nanoparticles, techniques for isolation, identification and characterization of biometabolites and its pharmacological evaluation.

Keywords: *Withania somnifera*, Nanoparticles, Pharmacological Significance

Introduction :

Nature is a Repository of diverse vegetation which possesses significant active bio-molecules having the capability to prevent incurable disease in humans. In the past, mankind used the plant material as medicaments in society for thousands of years. plants were the convention means of treatment for various ailments around the world specially in countries like China, Japan, Egypt, Brazil and India (Jamshidi-Kia, Lorigooini , Amini-Khoei, 2018).Factually, Maximum of population is depend on plants as traditional alternative of medicine in developing as well undeveloped and poor countries. Naturally Synthesized drugs like Aspirin, artimesinin, serpentine, colchicine, digoxin, ephedrine, morphine, physostigmine, Z guggulsterone, pilocarpine, reserpine, taxol, tubocurarine, paclitaxel, and vinblastine are some of the medications synthesized from plant extracted natural compounds (Dar, Shahnawaz , Qazi., 2017). Therefore, the Plant derived compounds now emerging as future medicine and will positioned as significant source natural remedy with minimum guarantee of side effects, is required using upcoming technologies.

In the past years, Nanotechnology and nanotools have emerged as potential tool in the area of technology and draw much attention due to their broad range of applications in physics, chemistry, biology, material science, and medicine (Karuppiah & Rajmohan: 2013). Application of metal like silver, gold, and copper in the synthesis of nanoparticles have been used for diagnosis and treatment of various disease because of their catalytic, optical, electronic, antimicrobial, and magnetic charaterstics (Zayed, Eisa, Shabaka: 2012). Physical and chemical methods like as electrochemical reduction and thermal evaporation in the synthesis of silver nanoparticles (AgNPs) methods are found time-consuming and unfavourable to mass production ((Jagtap & Bapat, 2013; Tan , Wang , Jiang & Zhu, 2002; Devaux , Laurent & Rousset,1993). More over the synthesis of nanoparticles, chemicals used are found environmentally hazardous. Thus a new method is in need that could avoid all the drawbacks as mentioned above, could be the ideal choice for preparing AgNPs. Some time before, it has been shown that some plant extracts can be synthesized in a highly controlled and hierarchical assembly, which shows the development of a reliable and appropriate ecofriendly process for metal nanoparticle synthesis. (Geethalakshmi & Sarada, 2013;. Vijayakumar , Priya , Nancy , Noorlida& & Ahmed , 2013; Jain , Daima , Kachhwaha & Kothari,2009; Vilchis-Nestor, Sanchez-Mendieta, Carnacho-Lopez, Gomez-Espinosa, Camacho-Lopez & Arenas-Alatorre ,2008; Abu Bakar, Ismail & Abu Bakar,2007; Gregory, Selvakesavan, Franklin, Sarmento & Dias,2014).

Plants derived natural drugs are the efficient source of therapeutic remedies for the treatment and prevention of number of sickness. Therefore herbal drugs have been emerged as a potential source for novel bioactive compounds. A great biodiversity among plants make them significant resource to discover new and novel compounds either themselves as whole drugs or precursor molecules for drugs with different mechanism of action. Medicinal plants comprised of several diverse class of phytochemicals or secondary bio metabolites that works independently, additionally or in synergy to

improve health (Srinivasahan & Durairaj, 2014). Plant extracts from different plant parts may have different modes of action to cure diseases. These plant derived extracts can also be applied as food preservatives (Bernhoft, 2010).
The quantity of phytochemicals is found different in different parts of the same plant or in different plants. Present metabolites such as alkaloids, flavonoids, saponins, terpenoids, steroids, phlobatannins, glycosides, tannins, etc is responsible for e therapeutic efficacy of plants. Present secondary metabolites are potential therapeutic compounds are also well-known for curing one or other diseases. Alkaloids are comprised off antispasmodic, antimalarial, analgesic, diuretic activity. Terpenoids are reported to have antiviral, anthelmintic, antibacterial, anticancer, antimalarial, anti-inflammatory properties also efficient in inhibition of cholesterol synthesis and possess insecticidal properties therefore useful for storing of agricultural products. Saponins a phytochemical compound imparts anti-inflammatory, antiviral activity along with plant defence and cholesterol reducing property. Phlobatanins shows astringent properties. Glycosides also have antifungal and antibacterial properties. Phenols and flavonoids are known for their antioxidant, anti-allergic, antibacterial propeties (Padalia & Chanda, 2015; Moteriya , Satasiya & Chanda, 2015).
Recent reports on the phytochemical investigation of different plant parts from the same or different plants is carried out to explore the particular activity of that plant part and it can also help one to decide the part(s) to be chosen for any synergistic evaluation. Phytochemicals study is preferred before the random selection of plant.

Presently, Green nanotechnology is now an important and advanced tool. Recently significant focus is being given to green synthesis to full fill primary objective of production of eco friendly, nanotechnology-based products that could be safer for all human being with sustainable commercial viability. The green syntheses of metal based nanoparticles are now getting a great attention because of their unusual optical, chemical, photochemical, and electronic properties. The green synthesis of nanoparticles is an emerging a branch of nanotechnology (Roy & Barik, 2004). The green synthesis approach using plant extracts has got special attention comparatively to chemical and physical methods and even the use of microbes. Green synthesis is a favourable method for metal based synthesis of nanoparticles due to requirement for aseptic conditions is not mandatory.

Now a days, Multi drug resistance pathogenic and opportunistic microorganisms showing a big threat against synthetic antibiotic which necessitate the search and development of new drugs and drug target. In the past decades, new formulation has been developed and applied by the pharmaceuticals sector, but these formulations has not found efficient against multidrug-resistant bacteria (Conlon, Kolodziejek & Nowotny, 2004). Moreover the green synthesis, formed nanoparticles have shown antimicrobial activities, which is used as the potential breakthrough for the development of antibiotic production.

The previous findings suggested that silver derivatives shows effective antimicrobial activity, however, the mechanism of its bioactivity is not known. Some studies exhibited that of silver-sulfur granules adheres on the microbial cell wall which inactivates essential enzymes by forming complexes with the catalytic sulfur of thiol group in cysteine residues.

Medicinal plant Withania somnifera, well-known as ashwagandha in Sanskrit in India, is a perennial plant belonging to the order *Solanaceae*. The plant is comprised of remarkable medicinal properties. *Withania somnifera* has been found effective in the treatment of burns, wounds, and skin disorders (Grierson & Afolayan, 1999). Roots of *Withania somnifera*, impats the significant pharmacological effects due to the presence of anolides, a group of steroidal lactones. Its leaves have great importance in Ayurvedic and Unani systems for the treatment of tumors and tubercular glands. The biological activities of *W. somnifera* are anxiolytic-anti-depressive (Bhattacharya, Bhattacharya, Sairam & Ghosal, 2000), antifungal (Dikasso, Makonnen, Debella, Abebe, Urga & Makonnen, 2006) , antimalarial (Girish , Machiah , Ushanandini, Harish Kumar, Nagaraju & Govindappa, 2006) apoptotic (Senthil, Ramadevi, Venkatakrishnan, Giridharan, Lakshmi & Vishwakarma,2007). Many findings elucidated the physicochemical and pharmacological properties of *W. somnifera* .

W. somnifera is a medicinal plant of tropical countries, including India, further more this plant is the potential source of bioreductant and stabilizers. The current study was expected to synthesis of silver nanoparticles using aqueous root extract of *W. somnifera* as reducing agent against silver nitrate and also studied their antimicrobial activity against bacterial and fungal pathogens.

Plant Description

The genus *Withania*, belongs to the family Solanaceae which comprises 26 species. *Withania* is widely distributed from the southern Mediterranean to the Canary Islands to south and east Africa and some parts of Asia, including India. *Withania somnifera* (L.) Dunal (WS) and *Withania coagulans* (Stocs.) Dunal (WC) species are found in India. The *Withania somnifera*, commonly known as Ashwagandha, winter cherry and Nagouri (Sangwan, Chaurasiya, Misra, Lal, Uniyal, Sharma, Sangwan, Suri, Qazi andTuli. 2004; POWO, 2022).
The *Withania somnifera* plants are erect, branching shrubs that grow up to one meter tall with simple petiolate, ovate, exstipulate, whole, acute, and glabrous describe the leaves. Flowers orientation is straightforward, short-pedicelled, gamosepalous, and persistent with five sharply lobbed sepals. Gamopetalous corolla has five spreading or recurved lobes that are acutely pubescent and greenish-yellow; epipetalous stamens have petals at the base. Slender filaments are inherent and have round anthers. A tiny, bulging ovary called the gynoecium syncarpous is surrounded by a long, thin style (Mir, Koul, Kuar, Sharma, Kaul and Soodan, 2012; Hepper, Hawkes, Lester, Nee and Estrada, 1991; Kite, Sivarajan and Balachandran, 1995; Kothari, Singh, Vijay Kumar and Singh, 2003). Roots consist of more useful chemical compounds in *Withania somnifera* than other parts.
India's production is substantially lower (1500 tons), but the domestic market is promising. The roots can be harvested 180 to 210 days from the seeding date. The entire plant is pulled up, and the aerial part's roots are removed. The maximum yield for cultivation is often 3 to 5 quintals per hectare.
Withania somnifera grows in arid and subtropical climates. Presence of a majority of bioactive compounds, which makes this plant is tough and drought-resilient. These properties of plant offer a monopoly in many regions of India, especially in Madhya Pradesh (Mir, Koul, Kuar, Sharma, Kaul and Soodan,

2012). It grows in arid parts in subtropical regions like Rajasthan (Nagour), Punjab, Haryana, Uttar Pradesh, Gujarat, Maharashtra, and Madhya Pradesh (Sangwan, Chaurasiya, Misra, Lal, Uniyal, Sharma, Sangwan, Suri, Qazi andTuli. 2004; Chopra, Nayar and Chopra, 1980; Nadkarni, 1982; Uddin, Samiulla, Singh, Jamil,2012).

This plant's demand is rising worldwide, this led the commercial cultivation, and few varieties have been released. Pure line selection was used to create these variants from the local cultivars. Since species have been cultivated for over a century, systematic breeding techniques have become crucial (Manivel, 2010; Deore and Manivel, 2014). Berries with diverse colors are now available with both annual and perennial growth habits since both natural (perennial) and farmed (annual) types are easily crossable (Kaul, Kumar and Sharma, 2005).

Extraction And Fractionation Techniques

Withania somnifera consists of a variety of bioactive metabolites, including alkaloids, withanolides, and flavonoids, which are accountable for their therapeutic properties. Different extraction techniques are applied to extract these biometabolites from plant material. Applied Extraction techniques play a vital role in extracting biometabolites from *Withania somnifera*. The selection of extraction technique depends on a variety of factors, like type of bioactive compounds to be extracted, the desired purity of the extract, and the intended use of the extract. Most common extracting techniques are the Reflux extraction; Soxhlet extraction, ultrasonic extraction, super- critical fluid extraction, maceration and microwave-assisted extraction. Application of these extraction techniques has facilitated the isolation of bioactive compounds from *Withania somnifera*, which can be employed for various therapeutic intentions.

Extraction of desired bio metabolites includes the use of suitable solvents and extraction techniques (Mohammad Azmin, Abdul Manan, Wan Alwi, Chua, Mustaffa and Yunus, 2016).The type of extraction process having an impact on the extracted bioactive compounds during herbal processing. The Selected extraction technique should be fast, modest, harmless to the ecosystem, reproducible, and high-yielding (WHO, 2022).

Previous Conventional methods used for extraction of phytochemicals from *Withania somnifera* plant include maceration, infusion, reflux, and Soxhlet extraction, which are based on the diffusion of the solvent into plant cells, bioactive compounds solubilization within the plant matrix, and the diffusion of bioactive compounds containing solvents from plant cells (Harbourne, Marete, Jacquier and O'Riordan, 2013; Ngo, Lau and Chua, 2018).

A maceration technique for extraction is based on soaking the powdered or crushed plant material in a solvent for a long time. During this process, the used solvent gradually dissolved the herb's active components. This method is basically used for extraction of delicate herbs and for bioactive compounds that are heat-sensitive (Dhanani, Shah, Gajbhiye, and Kumar, 2017). Maceration extraction is also preferred technique for extracting *Withania somnifera* as a perspectives of preservation of the heat sensistive phytochemicals and antioxidants present in the plant (Tsaltaki, Katsouli, Kekes, Chanioti and Tzia,2019; Ezez, Mekonnen and Tefera, 2023). Supercritical fluid extraction and pressurized liquid extraction are the modern techniques of extraction are widely used because of their of their superior performance in terms of high output, self-sufficiency and pricing (Handa,2008; Harbourne, Marete, Jacquier and O'Riordan, 2013; Putnik, Lorenzo, Barba, Roohinejad, Rezek Jambrak, Granato, Montesano and Bursac Kovacevic,2018). The Assess the variation in bioactive compound withanolide content for seasonal variation and effect of temperature variation using the New Millennium Indian Technology Leader- ship Initiative variety (NIMTLI-118) of *Withania somnifera (*Srivastava, Sangwan, Tripathi, Mishra, Narnoliya, Misra and Sangwan,*)*. Previous research suggests that the *Withania somnifera* grown in the winter has more withanolide content than the shrub grown in the summer (Mishra, Bose, and Sangwan, 2020). Therefore, temperature and environment significantly affects the withanolide content (Hatfield, Prueger, 2015).

Reflux extracting method is based on the boiling of herb in a solvent, later condensation and recycling back into the boiling vessel. Several repetition of this process makes the solvent to extract the active compounds from the herb. This makes the Reflux extraction is a popular method for extracting WS as it can efficiently extract the desired components and can be easily scaled up for larger batches (Dhanani, Shah, Gajbhiye, and Kumar, 2017; Ezez, Mekonnen and Tefera, 2023). It involves recycling the solvent for extraction and provides batch wise or continuous extraction setup as required. While it takes a longer cycle time for the complete extraction, the economic feasibility and scalability make it a widely accepted technique in large processing platforms. Another key advantage is that it ensures the complete extraction of actives from the plant material. So, loss of active compounds can be diminished.

A study showed that macearation of shade-dried and ground material of *Withania somnifera* leaves, stems, and roots with methanol for 14 days. The methanol extract of leaves gave the higher yields as compared with stem and root extract. In the same way, flavonoid content was higher in leaves (43.51 ± 0.346 mg/g) than in stem (42.82 ± 1.189 mg/ g) and root (39.13 ± 0.607 mg/g) extract. Furthermore, anti adipogenic withanolide was extracted from the root of *Withania somnifera* using reflux extraction with 80% aqueous methanol (Lee, Yoo, KangLee, Kim, Yu, KimJang, Pang and Kim, 2021). 19% and 22% yield was obtained when the dried root and fruits of plant *Withania somnifera* were homogenized separately at room temperature with methanol: water (8:2), respectively (Orabi, Zidan, Sakagami, Murakami, Ali, Alyami, Alshabi and Matsunami, 2022). Therefore, changes in bioactive levels according to taken plant parts make it an significant selection standard for studies. Execution of Any combinations without considering individual profiling will impact the accuracy and precision of the use.

A modern and sophisticated technique, Supercritical fluid extraction (SFE) uses supercritical fluids such as carbon dioxide as the solvent. Supercritical fluids consist of the unique properties of liquids and gases, making them ideal solvents for extracting bioactive compounds from WS. SFE is a selective and efficient method of extraction that results in a high-purity extract (Balkrishna, Nain, Chauhan, Sharma, Gupta, Ranjan and Varshney, 2020).

A 13% yield of faaty acid is obtained from Pulverized and dried *Withania somnifera* seeds when extracted with food-grade liquid carbon dioxide with backflow pressures of 450/80 and 555/40 bar/degree Celsius and CO2 flow of 60 g/min for 22 h.This extraction technique is also present a cleaner substitute for extraction of bioactives from plant material. The skilled operational setup is the basic need of the technique.

Two Previous extraction techniques (A. maceration and B. Soxhlet) and two modern extraction techniques (C. micro- wave-assisted extraction and D. subcritical water extraction) were compared using the raw material of WS, leaves, and roots.maceration gave 20.8% comperatively to Soxhlet (25.7%) when used as traditional procedures, on the other hand modern approaches have higher extraction yields (C. micro- wave-assisted extraction 30.2% and D. subcritical water extraction 65.6%) and have higher content (Bhatia, Bharti, Tewari, Sidhu and, Roy, 2013)).

Total flavonoids are main bioactive compounds of antioxidant activity; when the root of the plant is taken and extracted with different solvents at room temperature for 48 h. During extraxtion, it was found that the methanol extract (5.8%) gave a higher yield which was followed by water extract (4.2%), chloroform extract (0.7%), acetone extract (0.65%), and hexane extract (0.4%). The estmated total flavonoid content is also found higher in methanol extract (105.09 ± 8.5 mg QCE/g) as compared to other extracts, and the antioxidant activity is also higher.

The dried and pulverized roots of *Withania somnifera* were extracted with hot water, HPLC grade methanol, ethanol, and ethyl acetate using an orbital shaker and mechanical agitation and later the extract from each solvent is evaporated at 45−50 °C using a freeze-dryer and stored at room temperature. The high yield is obtained for methanolic extract (11.08%), followed by the aqueous solvent extract, ethanol extract (5.92%), and ethyl acetate extract (0.75%) (Kumar, Bouic and Rosenkranz, 2021; Mishra, Chaurasia and Srivastava, 2020).

The root material of *Withania somnifera* is extracted in water, a mixture of methanol, chloroform, and water (12:5:3), acetone, or aqueous methanol for 48 h at 40 °C (1:1). Aqueous, acetone, hydroalcohol and methanol−chloroform−water are the different extracts that are formed after the concentrates are filtered and evaporated at 40 °C. The hydromethanolic extract, 16.82%, had the highest yield comparatively to other extract (Orabi, Zidan, Sakagami, Murakami, Ali, Alyami, Alshabi and Matsunami, 2022).

The required polarity of the bioactive to be extracted is mainly based on the solvent polarity. Higher yields ensure efficient extraction process, while the concentration levels of desired bioactives compounds also need to be considered.

Previously, the hydroethanolic extraction of Withania *somnifera* roots extracted with an average yield of 15.40% (Prabu, Panchapakesan and Raj, 2013). The methanolic extraction of collected fresh unripe fruits of *Withania somnifera* at 30 °C, gave a yield of 1.54%, and later, it was subjected to liquid− liquid partitioning with hexane, dichloromethane (DCM), and ethyl acetate in a specific order. The highest yield was noticed for methanol layer (37%), followed by the DCM (3.2%) layer, the ethyl acetate layer (2.75%), and the hexane layer (1.46%) (Abutaha,2015). Moreoverin another extraction process of the Withania *somnifera* leaves, with 80% aqueous methanol and then exposed to liquid−liquid partitioning with specific solvents such as chloroform and butanol. The findings suggested that the butanol layer (26.48%) produces higher yields than the other parts (Tessema Desta, Andargie Ferede, Sisay Zewdu, andAdela Alemu, 2022).

During soxhlet extraction, *n*-hexane used as defatting and liquid partitioning with diethyl ether are applied to extract the leaves. When the leaves and roots of *Withania somnifera* were extracted using n-hexane, followed by methanol−water (90−70%, MeOH) at 35 °C. After liquid−liquid partitioning of the methanolic water layer with $CHCl_3$ and *n*-BuOH, the biometabolite variety was separated into four fractions of varying polarity (*n*-hexane, aqueous methanol, chloroform, and *n*- butanol). The amount of biometabolite in leaves was significantly found higher than in roots, mainly in the aqueous methanolic fraction (Chatterjee, Srivastava, Khalid, Singh, Sangwan, Sidhu, Roy, Khetrapal, and Tuli, 2010).

Previous presented data suggested that variables such as extraction solvent, techniques employed, and plant part used significantly affect the yield and assay. Although techniques such as MASE and UASE have verified promising results, however these techniques lacking the economic and practical viability for large-scale operations when compared to the conventional extraction mode. A further development is needed to overcome the limitations of traditional methods, to make them viable.

Analytical Techniques

Recent advancements in phytoanalytical research have led the researchers to identify, characterize, and quantify the biometabolites like withanolides and their derivatives sourcing from *Withania somnifera* using various chromatographic techniques that include TLC, HPTLC, HPLC-PDA, GC-MS, and LC-MS/MS (Chatterjee, Srivastava, Khalid, Singh, Sangwan, Sidhu, Roy, Khetrapal, and Tuli, 2010; Dhanani, Shah, Gajbhiye, and Kumar, 2017; Orabi, Zidan, Sakagami, Murakami, Ali, Alyami, Alshabi and Matsunami, 2022; Kumar, Bouic and Rosenkranz, 2021; Abutaha, 2015).

The thin layer chromatography (TLC) analysis is a versatile technique that is being used to identify the withanolide profiling in the *Withania somnifera* extract.High-performance thin-layer chromatography (HPTLC) is a modern version of TLC used for the qualitative analysis and identification of multiclass metabolites present in root and fruit hydroalcoholic extracts (Orabi, Zidan, Sakagami, Murakami, Ali, Alyami, Alshabi and Matsunami, 2022).

HPLC is an advanced and accurate chromatographic technique used to identify and quantify bioactive compounds present in *Withania somniferous* extracts. In a number of studies, biometabolites withanolide derivatives and withanosides have been identified and quantified using HPLC PDA methods. The refractive index detector is the choice when one needs to detect analytes with restricted UV absorption such as alcohols, sugars, carbohydrates, fatty acids, and polymers. It has also been reported that these multiclass compounds are identified and quantified with ELSD detection (Chaurasiya, Uniyal, Lal, Misra, Sangwan, Tuli and Sangwan,2008).

Gas chromatography−mass spectrometry (GCMS) analysis was performed with ESI mode at 70 eV to generate mass spectra. The GC−MS running conditions were the same as those mentioned earlier. Biometabolite was quantified using its percentage peak area that appeared at the total ion chromatogram in GC−MS analysis (Chatterjee, Srivastava, Khalid, Singh, Sangwan, Sidhu, Roy, Khetrapal, and Tuli, 2010).

Liquid chromatography coupled with PDA and MS detectors can be used to obtain essential information about WS extracts, such as probable phytocompounds present in the extract. Based on their mass, m/z transitions (MS/MS fragments), UV maxima of each peak, and relative retention periods, compounds can beidentified using online databases and literature.

Another technique, ultrasound-assisted solvent extraction (UASE) is a nonthermal extraction method that uses high- frequency sound waves to disrupt the cell walls of the plant material, thereby facilitating the release of bioactive com- pounds. The plant material is immersed in a solvent, such as ethanol or water, and subjected to ultrasonic waves. Ultrasonic extraction is a rapid and efficient method of extraction that results in high yields of bioactive compounds (El Maaiden,Bouzroud, Nasser, Moustaid, El Mouttaqi, Ibourki, Boukcim, Hirich, Kouisni and El Kharrassi, 2022; , S. C.; Ishida, Tamura, Wada, Iitsuka, Garg, Kim, Gao, Nakai and Okamoto, 2016).

Pharmacological Significance

5.1 Antimicrobial activity

Recent studies on The leaves and roots of *W. somnifera* exhibited antimicrobial activity. Leaf extracts with concentrations of 6.25 mg/ml and 12.5 mg/ml significantly inhibited the growth of five Gram-negative pathogenic bacteria (*Escherichia coli, Salmonella typhi, Citrobacter freundii, Pseudomonas aeruginosa* and *Klebsiella pneumonia*) (Alam, Hossain, Mottalib, Sulaiman, Gan , Khalil, 2012). phytochemicals flavonoids and alkaloids Isolated from *W. somnifera* show antimicrobial activity against *Enterobacter aerogens, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Raoultella planticola* and *Agrobacterium tumefaciens* when used in concentration of 0.039 mg/ml (Singh and Kumar, 2011; Singh and Kumar, 2012). In another study, crude extract of leaves of *W. somnifera* was applied against clinical pathogens *Staphylococcus aureus, roteus mirabilis Streptococcus mutans, Streptococcus sobrinus* and *Salmonella paratyphi* B, 100 µl of extracts (100 mg/ml) was successfully inhibited the growth of all the pathogenic bacteria (Al-Ani, Hadi and Nazar , 2013; Pandit, Chang and, Jeon., 2013). The antimicrobial potency of *W. somnifera* was thought to be found very effective against pathogens due to presence of antimicrobial compounds.

5.2 Hepatoprotective activity

Several studies were carried out to evaluate the hepatoprotective significance of *W. somnifera. W. somnifera* conventionally used for various treatments, its application in the hepatoprotective activity was also considered significant. Several findings have given numerous evidences that a dose 500 mg/kg of W. *somnifera* significantly reduces the elevated biomarkers (aspartate aminotransferase, alanine transaminase, alkaline phosphatase, and Bilirubin) in experimental animals modal when injected with hepatotoxic dose of paracetamol. It drastically decreases the lipid peroxidation, enhances glutathione content, catalase, glutathione reductase and glutathione peroxidase activity in
Liver (Malik, Pandey and Dogra, 2013; Sabina, Rasool , Vedi , Navaneethan , Ravichander , Parthasarthy and Thella , 2013). These study studies suggestes the hepatoprotective activity of *W. somnifera*.

5.3 Antidepression and antianxiety activity

Now a day's major population is in arrest of anxiety and depression worldwide. Howver from ancient times, ayurveda is curing such problems by using the roots of *W. somnifera* the treatment of anxiety and depression. Previos study reported the anxiolytic-antidepressant potential of *W. somnifera* and its glycowithanolides (Bhattacharya, Sairam and Ghosal, 2000). Present study also also supports the remedial properties of *W. somnifera* for depression and anxiety disorders. a very recent study suggests, that found that a dose of 40 mg/kg of *W. somnifera* significantly reduces the depression in various experimental models (Jayanthi, Prathima , Huralikuppi , Suresha and Dhar, 2012). Clinical trials on healthy volunteers also revealed that aqueous extracts of *W. somnifera improve* the psychomotor performances in anxiety and depression (Pingali. Pilli and Fatima, 2014).

5.4 Antiinflammatory and antiarthritic activity

Research studies suggested that *W. somnifera* exhibits effective antiarthritic and anti-inflammatory activities. Antiinflammatory activity has been quality to bio active steroids, withaferin-A is a major component present in plant *W. somnifera*. Recent studies on dose experimental animals also revealed the antiarthritic and anti-inflammatory activity of *W. somnifera* (Gupta and Singh, 2014). Roots of *W. somnifera* contains withanolides are also found effective in treatment of arthritic inflammation, cystic fibrosis inflammation and irritable bowel syndrome, by inhibiting NF-κB activation, inhibition of COX-2 generation, inhibition of endothelial cell protein C receptor through antioxidant effect and cytokines release, thus in turn causes depletion of inflammatory mediators (Ku, Han and Bae, 2014; Mulabagal Subbaraju, Rao Sivaramakrishna, DeWitt, Holmes, Sung, Aggarwal and Tsay, Nair, 2009; Oh and Kwon, 2009). *W. somnifera* and its bioactive withaferin-A down regulate the production of inflammatory mediators like prostaglandins, histamine, interleukins and cytokines (Gupta and Singh, 2014; Paval, Kaitheri , Potu , Govindan , Kumar and Narayanan, 2009). Bio active compound Withaferin-A has been shown to stimulates differentiation and growth of osteoblasts in menopausal osteoporosis and by bone injury, through increased expression of osteoblast-specific transcription factor and mineralizing genes (Khedgikar Kushwaha, Gautam, Verma, Changkija, Kumar, Sharma, Nagar, Singh, Trivedi, Sangwan, Mishra and Trivedi, 2013). Therefore all these mechanisms of *W. somnifera* show its antiinflammatory and antiarthritic activity, which makes it valuable for the treatment of various inflammatory disorders. Studies on several rodent models has been shown the analgesics activity of *W. somnifera* and thus suggested for various pain relieving therapies (Sabina et al., 2009; Shahriar, Alam and Uddin, 2014).

Conclusion

This review insights the significance of extraction techniques in the isolation of bioactive compounds present in *Withania somnifera,* including green synthesis techniques and also role of solvents as well as seasonal variations. This review also signifies the analytical techniques using in the identification and characterization of synthesized nanoparticles. On the basis of pharmacological perspective, present bioactive compounds withanolides and other phytoconstituents have the therapeutic potentiality against various diseases and antimicrobial properties. The benefits could be utilized for society.

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