FTIR Spectrum Analysis and Antimicrobial Activity of Roots and Leaves Extracts of Five Indian Medicinal Plants

Wajed A. Khan[1*], Faiyaz K. Shaikh[2], Sarwan W. Bradosty[3, 4]

[1]Department of Microbiology, Dr. Rafiq Zakaria Centre for Higher Learning and Advanced Research, Aurangabad, Maharashtra, India, kzaroon783@gmail.com

[2]Department of Biochemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India, faizbiochem@gmail.comv

[3]Department of Community Health, College of Health Technology, Cihan University-Erbil, Kurdistan Region, Iraq

[4]Department of Biology, Faculty of Arts and Sciences, Harran University, Sanliurfa, Turkey, sarwan.hamad@cihanuniversity.edu.iq

Abstract-The purpose of the present study was to find out the bioactive phytochemical content, their functional groups and antimicrobial activity of five selected Indian medicinal plants- *Rauwolfia serpentine (*roots*), Tephrosia purpurae (*leaves*), Delonix regia (*leaves*), Withania somnifera (*roots*)* and *Asparagus racemosus (*roots*)*. The selected parts of plants were dried and the solvent extracts were prepared by distilled water extraction using Soxhlet apparatus. Phytochemicals were identified using primary screening test. Phytochemical analysis of plant extracts showed the presence of coumarins, steroids, saponins, phenolic compounds, flavonoids, alkaloids and sterols. FTIR analysis between the scan ranges of 400-4000 cm-1 is carried out. The FTIR spectroscopic investigation revealed that various functional groups like ethers, amines, phenol, ketone, carboxylic acids, alcohol, alkanes, aromatic compounds, etc. were identified. Antimicrobial investigations were done by using agar well diffusion method. It showed that the root extracts of *Rauwolfia serpentina* exhibit highest zone of inhibition against all test organisms- E. coli, S. aureus, *S. typhi* and *Bacillus*. From the results it is confirmed that these selected plants possess potential of bioactive compounds which are responsible for the biological activities that are useful for natural health.

***Keywords*- antimicrobial activity, medicinal plants, FTIR analysis, phenolic compounds, flavonoids, alkaloids**

INTRODUCTION

The word "environment" comes from the French word "environ."It encompasses anything outside the plant that has an impact on the plant's life, either directly or indirectly. This is an essential component of the planet's ecosystem. The term "environmental factor" refers to each component of the environment. Temperature, soil moisture, soil nutrients, light, air pollution, humidity, soil structure, and pH are all conditions that plants prefer to grow in. Although these elements affect all plants, they are frequently cultivated or kept under cultural components (fertilization, irrigation, pesticide spraying) that can have a significant impact on their growth. According to the WHO, traditional forms of medicine, mostly plant-based, are used by over 80% of the world's population to satisfy primary health care needs. In India, the harvesting and processing of medicinal plants and plant products contributes a significant portion of the national economy each year, providing both full-time and part-time jobs.

One of the most important sources of medications is plants. Plants have been used as remedies from the beginning of time. The Rig-Veda references to the healing powers of various herbs appear to be the first records of plant use in medicine in India. Medicinal plants are widely used in two types of health management systems around the world: traditional medicine and modern medicine. Traditional medicine is divided into two streams: (1) local or folk or tribal medicine, and (2) codified and organised Indian medicine such as Ayurveda, Siddha, and Unani, among others. Despite advances in modern medical and pharmaceutical research, the usage of therapeutic plants has become an important part of daily living over the years. In India, there are over 3000 plant species with therapeutic potential. The use of therapeutic plants is mentioned in the Rig-Veda (3700 B.C.). Herbs are used in Ayurveda, Unani, Siddha, and Homeopathy, among other ancient medical systems. Plant-based medicine is expected to be the primary source of health care for 40% of the world's population. Medicinal plants provide low-cost and safe health-care options in India.

Plants have been used to preserve and promote human body wellness since prehistoric times. Plant consumption was thought to improve health status in the past, possibly by raising energy, reducing disease symptoms, or curing a sickness. As a result, the helpful plant was given the name medical plant. Fortunately, the Indians recorded useful information on medicinal plants such as their common name, medicinal use, and method of preparation in the Ayurvedic book, the Egyptians with Hippocrates' Greek Epidemic Book III, and the Chinese with the Chinese Nei Ching.[1] At the same time, elders practised and passed on their knowledge of medicinal herbs to the next generation.

Complementary and Alternative Medicine (CAM) is the modern name for traditional medical practise. A range of medical and health-care systems, practises, and products that are not now considered part of traditional medicine are referred to as complementary and alternative medicine.[2] To be more exact, complementary medicine is used in conjunction with traditional medicine or therapy, whereas alternative medicine is utilised independently of traditional medicine.

Ancestors employed medicinal herbs to heal minor ailments before the invention of modern medicine. Traditional medicine's knowledge and practises, which have been used for hundreds of years and are founded on beliefs and observations,

DOI: http://doi.org/10.24086/biohs2022/paper.799

should not be thrown away. Some medicinal herbs that were employed in ancient times are now used as modern-day medications, according to research.[2]

Metformin, an anti-diabetic medicine made from a bioactive component derived from French lilac (*Galega officinalis*), is a notable example.[3] Herbal medicine was used twice to three times more than conventional drugs, and 80 percent of the world's population, particularly in underdeveloped nations, still relies on plant-derived medicines for health maintenance and disease treatment (World Health Organization, 2002). The strong demand for medicinal plants stems from their long history of use and local faith in their power to heal. The restorative powers of medicinal plants, on the other hand, must be found, refined, and confirmed using scientific procedures.

The current investigation was aimed to explore bioactivie composition of well-known medicinal plants in India. We have explored roots extracts of *Rauwolfia serpentine*, *Withania somnifera* and *Asparagus racemosus* whereas leaves extracts of *Tephrosia purpurae* and *Delonix regia* for antimicrobial activity and FTIR analysis.[4]

I. MATERIALS AND METHODS

Collection of Plant Material

Five medicinal plants *Rauwolfia serpentine*, *Tephrosia purpurea, Delonix regia, Withania somnifera* and *Aspar*agus *recemosus* were collected. The roots of *Rauwolfia serpentine*, *Withania somnifera*, *Asparagus racemosus* and the leaves of *Tephrosia purpurae* and *Delonix regia* were shade dried at room temperature in a clean environment to avoid contamination for few days.[5]

Preparation of Plant Material

The dried roots and leaves are cut into small pieces. These pieces were then grinded in an electric grinder. The powdered plant materials were used as an extract for further study.

Extraction of Plant Material

The extraction was performed using a Soxhlet apparatus in the normal way at the boiling point of the solvent used. Solvent used for extraction is Distilled water and ethanol. 50 g of powdered plant material (roots and leaves) were extracted with 500 ml distilled water. The extracts were then boiled till it gets condensed. They were then collected in tubes and stored at 4ºC for further investigation for phytochemical analysis by phytochemical tests, FTIR Spectrum analysis and for checking Antimicrobial Activity.[6]

Phytochemical Examinations

All of these plant species were subjected to a preliminary qualitative screening for phytochemicals using the following methods:

1- **Coumarins test**: 2 ml of extract was treated with 3 ml of 10% NaOH. The production of a yellow tint was seen, indicating the presence of coumarins.

2-**Leucoanthocyanins** were determined by allowing 5 mL of extract to react with 5 mL of isoamyl alcohol. The presence of leucoanthocyanins is indicated by the colour of the upper layer being red.[7]

3-**Steroids Test**: 1 ml of extract was dissolved in 10 ml of chloroform (Libermann Burchard Test). By the sides of the test tube, an equal volume of concentrated sulfuric acid was added to this mixture. The upper layer of sulfuric acid turns red, while the lower layer turns yellow with green fluorescence, suggesting the presence of steroids.[7]

4-**Saponins Test**: (Foam test) 2 ml of extract was placed in a test tube, and 6 ml of distilled water was added. The mixture was then forcefully shook. The presence of saponins was determined by the persistence of foam.[7]

5-**Quinones Test**: 1 mL extract + 2 mL dilute NaOH = 5 mL Quinones Test The presence of quinines is confirmed by the formation of blue green or red coloring.[8]

6-**Precipitate test for phlobatannins**: 2 mL extract was added to 2 mL 1 percent aqueous hydrochloric acid and the mixture was heated. The existence of phlobatannins was established by the formation of a crimson precipitate.[8]

7-**Phenolic Compounds Test**: (Ferric chloride test) A few drops of the extract were treated with aqueous ferric chloride at a concentration of 5%. The presence of phenolic chemicals is indicated by the formation of a deep blue or black colour.

8-**Flavonoid Detection**: (Alkaline reagent test) 2 ml of extract was treated with a few drops of 1N sodium hydroxide solution and a strong yellow hue was seen. When dilute hydrochloric acid is added to the yellow colour, it turns colourless, showing the presence of flavonoids. [6,9]

9-**Alkaloids Test** (Mayer's Test) 2 drops of Mayer's reagent were added to 2 ml of extract. A positive test is indicated by the presence of white creamy precipitate.

10-**Test for Sterols**: (Salkowski test) 2 ml extract, 2 ml chloroform, and 2 ml concentrated H2SO4 were carefully mixed and agitated well. The chloroform layer was bright red, whereas the acid layer was bright greenish yellow.

FTIR Analysis

Fourier transform infrared spectroscopy (FTIR) spectrum analysis between the scan ranges of 400-4000 cm^{-1} is carried out.

Antimicrobial Screening

The bacterial cultures (*E. coli, S. aureus, S. typhi* and *Bacillus*) were grown in nutrient broth medium at 37ºC. After 4 hours of growth, each microorganism was uniformly spread on the surface of nutrient agar plates by a sterile spreader. Then holes were punched aseptically with a sterile well borer. The well present in the center of each plate was introduced by Streptomycin taking it as the standard antibiotic. Then the extract solutions were introduced in the remaining wells (extracts of *R. serpentina* and *D. regia* in the wells of four plates, extracts of *T. purpurae*, *W. somnifera* and *A. racemosus* in the wells of other four plates). These plates were then kept in fridge at 4ºC for 5 minutes and then they are kept for incubation at 37ºC for 24 hours. Next day the plates were observed for any antimicrobial activity.

DOI: http://doi.org/10.24086/biohs2022/paper.799

II. RESULTS AND DISCUSSION

The goal of this study was to identify the phytochemical characteristics, functional groups, antibacterial activity, and structural analyses of the traditional plants that were chosen.[4]

Phytochemical Analysis

The phytochemical studies revealed the presence of secondary metabolites like coumarine, saponins, flavonoids, alkaloids and sterols in the root extract of *R. serpentina*, the leaf extract of *T.purpurae* contains coumarine, saponins, phenolic compounds and flavonoids, the leaf extract of *D.regia* contains coumarine, saponins, phenolic compounds and sterols, the root extract of *W.somnifera* contains coumarine, saponins, quinones, flavonoids, alkaloids and sterols and the root extracts of *A.racemosus* contain saponins, alkaloids and sterol. (**Table 1**).

Table 1: Phytochemical analysis of selected medicinal plants extracts

Tests	*R. serpentine* (root extract)	*T. purpurae* (leaves extract)	*D. regia* (leaves extract)	*W. somnifera* (root extract)	*A. racemosus* (root extract)
1.Coumarin	+	+	+	+	-
2. Leukoanthocyanin	+	-	-	-	-
3. Steroids	+	+	+	+	+
4. Saponin	-	+	+	+	+
5. Quinones	-	-	-	+	-
6. Phlobatannins	-	-	-	-	-
7. Phenolic compounds	-	+	+	-	-
8. Flavonoids	+	+	-	+	-
9. Alkaloids	+	-	-	+	+
10. Sterol	+	-	+	+	+

The existence of secondary metabolites in plants causes biological effects in humans and animals, which is why they are used as herbs.[10] Coumarine has anticoagulant property. Saponins are used as anti-inflammatory, hypo- cholesterolemic, immune stimulating agent. Flavonoids have a wide range of therapeutic activities such as anti-hypertensive, anti-rheumatism, anti-diuretic, anti-oxidant, anti-microbial and anti-cancer properties. Alkaloids exhibited promising anti-diarrheal, anti-malerial, anti-inflammatory, anti-diabetic, vasodilatory and cure urinary disorders. Sterol has anti-cancer properties which helps against stomach cancer, colon cancer, rectal cancer and also have weight loss properties.[10]

FTIR spectrum analysis

The FTIR analysis of leaf extracts of *T.purpurae* and *D.regia* reveal the presence of branched alkane, cyclic ether, phenol, ketone, carboxylic acid, nitrile, 1˚ amine, aromatic phosphate and polyhydroxy compounds. Major peaks observed in *T.purpurae* and *D.regia* is 3334.92 & 3332.99 that indicates the presence of O-H hydroxy group (**Figure 1, 2**).

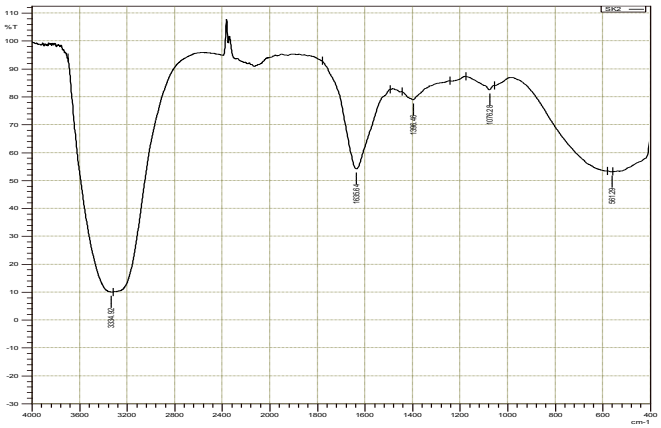

Figure 1. *FTIR Spectrum of T. purpurae*

DOI: http://doi.org/10.24086/biohs2022/paper.799

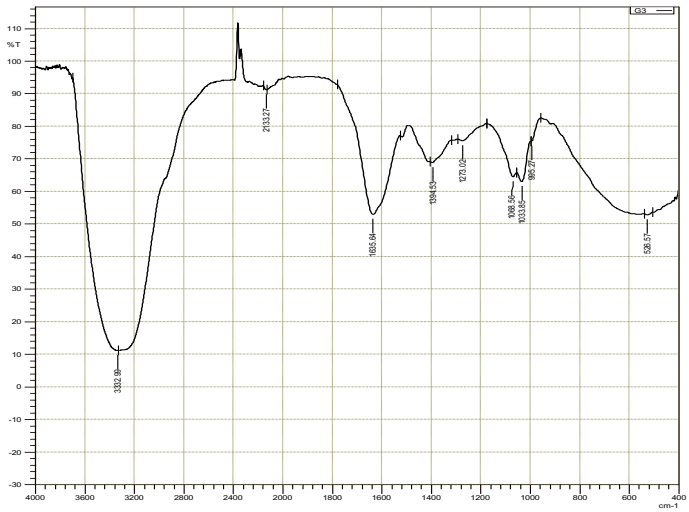

***Figure 2**. FTIR Spectrum of D. regia*

The FTIR analysis of root extracts of *R. serpentina*, *W. somnifera,*and *A. racemosus* reveal the presence of ketone, aromatic phosphate, lipids, proteins, cyclic ethers, carboxylic acid, phenol, straight chain alkane, branched chain alkane, primary amine, acetate, amide and polyhydroxy compounds. Major peaks observed in the root extract of *R.serpentina*are 3356.14 & 1045.42 that indicates the presence of O-H hydroxy and P=O phosphate group (**Figure 3**).

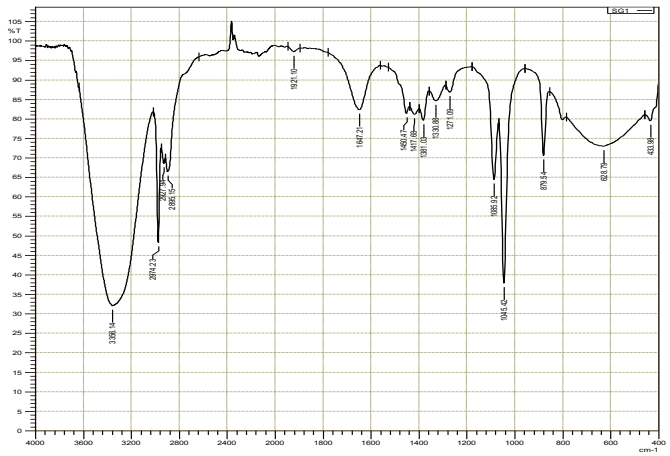

***Figure 3.** FTIR Spectrum of R. serpentina*

Major peak observed in the root extracts of *W.somnifera* and *A.racemosus* are 3336.85 & 3317.56 that indicates the presence of O-H hydroxy group (**Figure 4, 5**).

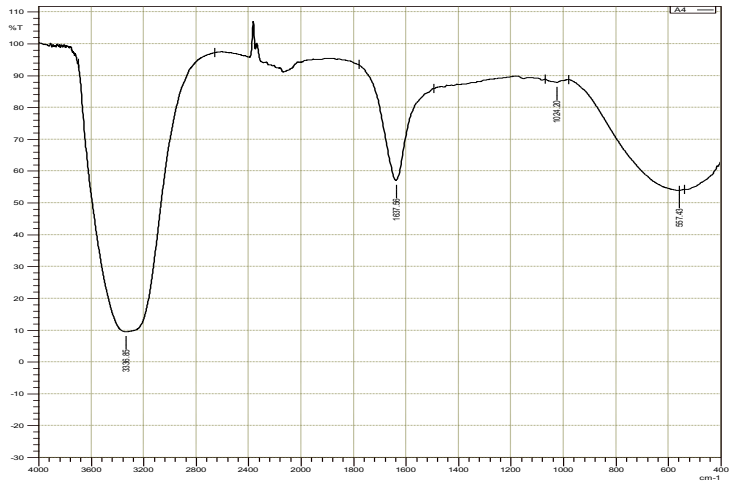

***Figure 4.** FTIR Spectrum of W. somnifera*

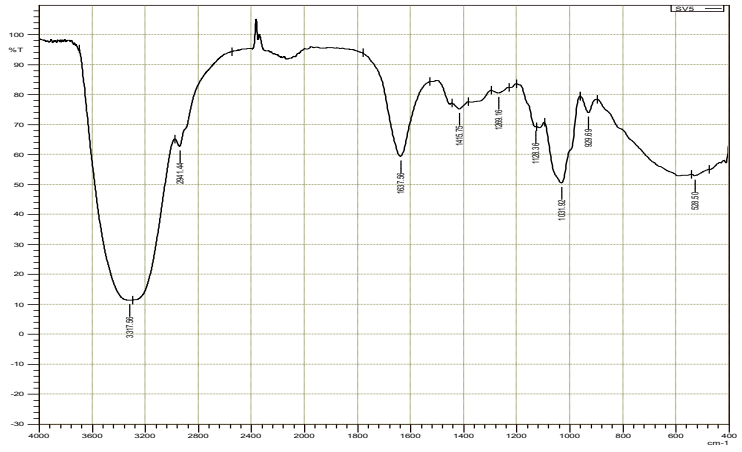

***Figure 5.** FTIR Spectrum of A. racemosus*

Ketone drugs are used in acne treatments and also has pharmaceutical value. Aromatic phosphates are used as dietary supplement and against urinary tract infection. Protein are anti-cancer and used to treat haemophilia and anemia. Cyclic ethers are used as anesthetic agents. Carboxylic acid, a significant pharmacological substance found in the medicinal plant, is used to treat ulcers, jaundice, headaches, stomatitis, fever, edoema, and rheumatic joint symptoms. Protein synthesis is dominated by amines and amides, and herbs are used to make herb oil and hair tonics.

Amines are also use as analgesic, anesthetic and congestants. Acetates are use in urine alkalinization, metabolic acidosis, as a drug for high BP and kidney disorder. Norethindrine acetates are used to cure abnormal bleeding in females. Alkanes are used as greenhouse gas and also have pharmaceutical value. Nitriles have anti-diabetic, anti-cancer and anti-psychotic properties.[11]

Antimicrobial Screening

The compounds were screened against four test organisms (*E. coli, S. aureus, S. typhi* and *Bacillus*). Only *R. serpentina* showed antimicrobial property against all four test organisms**(Table 2)**.Similar type of anti-microbial activity of

DOI: http://doi.org/10.24086/biohs2022/paper.799

Rauwolfiaserpentina, *Tephrosiapurpurae*, *Delonixregia*, *Withaniasomnifera* and *Asparagusracemosus* were tested against *Escherichiacoli*, *Staphylococcusaureus*, *Salmonellatyphi* and *Bacillus*. The root extract of R.*serpentina* inhibit the growth of all test organism **(Table 2).** The level of inhibition varies between the bacteria. Certain well known urinary tract infection and urinary disorder causing bacteria – *E. coli* and *S.aureus* were predominantly controlled by this crude extract of *R.serpentina*. .[12] Various diseases like typhoid, pneumonia, meningitis caused by *S.typhi* and *Bacillus* species can also be controlled by the crude extract of *R.serpentina*.[13] Hence the extracts can be used as natural anti-bacterial compound to prevent the infection of these bacteria.

Table 2: Antimicrobial activity of selected medicinal plants extracts

Sr. no.	Organism	Compound	Zone of inhibition (in cm)
1.	***E. coli***	i) Standard antibiotic (Streptomycin) ii) *R. serpentina* iii) *T. purpurae* iv) *D. regia* v) *W. somnifera* vi) *A. racemosus*	2.4 cm 1.9 cm - - - Negligible
2.	***S. aureus***	i) Standard antibiotic (Streptomycin) ii) R. serpentina iii) *T. purpurae* iv) *D. regia* v) *W. somnifera* vi) *A. racemosus*	- 2.8 cm - - Negligible Negligible
3.	***S. typhi***	i) Standard antibiotic (Streptomycin) ii) *R. serpentina* iii) *T. purpurae* iv) *D. regia* v) *W. somnifera* vi) *A. racemosus*	1.1 cm 1.5 cm - - 2.3 cm -
4.	***Bacillus***	i) Standard antibiotic (Streptomycin) ii) *R. serpentina* iii) *T. purpurae* iv) *D. regia* v) *W. somnifera* vi) *A. racemosus*	2 cm 1.9 cm - - - negligible

CONCLUSIONS

This study reports bioactive phytochemical content, their functional groups and antimicrobial activity of five selected Indian medicinal plants i.e. *R. serpentine*, *T. purpurae*, *D. regia*, *W. somnifera* and *A. racemosus*. Detailed biochemical characterization and antimicrobial potential of these extracts could be explore further is future prospective of this study.

REFERENCES

1. Vuksan, V., & Sievenpiper, J.L. (2005). Herbal remedies in the management of diabetes: lessons learned from the study of ginseng. Nutrition, metabolism and cardiovascular disease, 15(3), 149-60. https://doi.org/10.1016/j.numecd.2005.05.001

2. Marles, R.J., & Farnsworth, N.R. (1995). Antidiabetic Plants and Their Active Constituents. Phytomedicine, 2, 137-189. https://doi.org/10.1016/S0944-7113(11)80059-0

3. Kumar, D., Bajaj, S., & Mehrotra, R. (2006). Knowledge, attitude and practice of complementary and alternative medicines for diabetes. Public Health, 120(8), 705-11. doi: 10.1016/j.puhe.2006.04.010

4. Lethika, D.N., Santosh, K.S., Arun, A., & Deepak M. (2013). Fourier Transform Infrared Spectroscopy Analysis of few Medicinal Plants of Chattisgarh. India Journal of Advanced Pharmacy Education & Research, 3(3), 196-200.

5. Dwivedi, T., Chandra Kanta, Singh, L.R., & Sharma,I.P. (2019). A list of some important medicinal plants with their medicinal uses from Himalayan State Uttarakhand, India. Journal of Medicinal Plants Studies, 7(2), 106-116.

6. Soni A., & Sosa S. (2013)**.** Phytochemical Analysis and Free Radical Scavenging Potential of Herbal and Medicinal Plant Extracts**.** Journal of Pharmacognosy and Phytochemistry, 2 (4), 22-29.

DOI: http://doi.org/10.24086/biohs2022/paper.799

7. Bohren, C.F. and D.R. Huffman, (2004) "Absorption and scattering of light by small particles", Weinheim: Wiley. Xiv, 530 s.

8. Kohler, A., Sulé-Suso, J., Sockalingum, G.D., Tobin, M., Bahrami, F., Yang, Y., Pijanka, J., Dumas, P., Cotte, M., van Pittius, D.G., Parkes, G., & Martens, H. (2008). Estimating and correcting Mie scattering in synchrotron-based microscopic Fourier transform infrared spectra by extended multiplicative signal correction. Applied Spectroscopy, 62(3), 259-66. doi: 10.1366/000370208783759669.

9. Ghosh, S., Derle, A., Ahire, M., More, P., Jagtap, S., Phadatare, S.D., Patil, A.B., Jabgunde, A.M., Sharma, G.K., Shinde, V.S., Pardesi, K., Dhavale, D.D., & Chopade, B.A. (2013) Phytochemical analysis and free radical scavenging activity of medicinal plants *Gnidia glauca* and *Dioscorea bulbifera*. PLoS One, 8(12), e82529. doi: 10.1371/journal.pone.0082529.

10. Ven Murthy, M.R., Ranjekar, P.K., Ramassamy, C., & Deshpande, M. (2010). Scientific basis for the use of Indian Ayurvedic medicinal plants in the treatment of neurodegenerative disorders: Ashwagandha. Cent NervSyst Agents Med Chem, 10 (3), 238 – 246.

11. Sangwan, R. S., Chaurasiya, N. D., Misra, L. N., Lal, P., Uniyal, G. C., Sharma, R., Sangwan, N. S., Suri, K. A., Qazi, G. N., & Tuli, R. (2004). Phytochemical variability in commercial herbal products and preparations of *Withania* somnifera (Ashwagandha). Current Science, 86(3), 461–465. http://www.jstor.org/stable/24108744

12. Shah P., Modi H. A., Shukla M. D., & Lahiri S. K. (2014). Preliminary Phytochemical Analysis and Antibacterial Activity of *Ganoderma lucidum* collected from Dang District of Gujarat, India. International Journal of Current Microbiology and Applied Sciences, 3(3), 246-255

13. Gogte, V.M. (2001). Ayurvedic pharmacology and therapeutic uses of medicinal plants. Ayurveda Research Centre (SPARC), p. 836-839.

DOI: http://doi.org/10.24086/biohs2022/paper.799