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FT-IR, UV AND ANTIMICROBIAL ACTIVITY *WITH ANIA SOMNIFERA* AND *WITHANIA OBTUSIFOLIA*

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ABSTRACT

The present study was to compare the Phytochemical and Antimicrobial analysis of *W. somnifera* and *W. obtusifolia*. In the Ethanolic leaf and root extract of *Withania* species were screened for phytochemical compounds by qualitative and FT-IR, UV method. *Withania* species have been used for remedies of human pathogen because they contain therapeutic compounds. This research work was mainly focused to indicate on the identification of electron transition and the functional group of phytochemical constitute like wise FT-IR and UV spectroscopy

KEYWORDS: ***W. somnifera*, *W. obtusifolia*, Antimicrobial activity, FT-IR, UV-Visible spectroscopy analysis**

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INTRODUCTION

The genus *Withania* belongs to the family Solanaceae. Members of this family are mostly herbs and shrubs with about 70 genera and 2,000 species [1]. *W. somnifera* is considered to be one of the highly reputed medicinal plants and it is commonly called Aswagandha. It is known as Indian Ginseng. There are about sixty six species present in this genus and they are distributed throughout the world. In India only three species are found namely *Withania somnifera*, *Withania obtusifolia* and *Withania coagulans.* It is distributed in tropical and subtropical region like Rajasthan, Madhya Pradesh, Punjab, Himachal Pradesh, Jammu and Kashmir and Tamil Nadu. It is used as anticancer, anti-inflammatory, fertility increased, food for cattle and to make chutney[2]. The two species of *Withania* i.e. *W. obtusifolia* and *W. somnifera* were usually misinterpreted because of their co-existence in mixed natural populations. The application of morphological and anatomical study has proven to be immense assistance in interpreting problems related to identification of differences between the two species[3, 4]. The present research work aims at identification of functional group of the bioactive compounds from the dried leaves of two *Withania* species.

MATERIALS AND METHODS

(i) Plant collection

The whole plant materials and seed of *W. obtusifolia* and *W. somnifera* used in this study were collected from their natural habitats of Karudamangalam in Tiruchirappalli and Pulavarnatham in Tanjore districts.

(ii) Solvent extract

Ten grams of dried powdered materials (stem) were soaked separately in 50ml of the ethanol solvent with in a soxhlet apparatus for 72 hrs. The extracts were evaporated under vacuum and the residues were separately dissolved in the same solvent.

(iii) Medium for microbial growth

The nutrient Agar and Rose Bengal Agar medium were prepared and sterilized for bacteria and fungi respectively. Two fungal species namely *Aspergillus niger*, *Trichoderma viride* and three bacterial species such as *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumoniae* were obtained from Eumic Analytic Lab, Tiruchirappalli, Tamil Nadu. Both fungal and bacterial strains were used in agar well diffusion methods and the respective temperature at 37^0C for 48 to 72 hrs for mother culture[5]. The different leaf extracts were taken separately at various concentrations of 25, 50, 75 and 100µl. They were kept under incubation. After incubation the plates were observed in the zone of inhibition and the length (mm) of the zone was measured and tabulated.

(iv) FT-IR analysis

The FT-IR analysis of ethanolic extracts of plant samples by using BRUKER 1FS 66Umodel FT-IR and FT Raman spectrometer using KBr pellet and powder from respectively. The FT-IR was recorded in the range 400-4000cm^{-1}. The assignments were made assuming Cs point group symmetry; the various modes of vibration were identified and assigned. In the report, apart from the fundamental bands; some more bands found in the spectra are attributed to overlapping combinations and lattice vibration[6].

(v) UV Visible spectrum study

Nonlinear optical property of the plant sample has been tested by Kurtz powder technique. Its optical behaviour was examined by Ultraviolet-spectrum instrument model Lambda 35 and found that the crystal is transparent in the region between 380-900nm[7].

RESULTS AND DISCUSSION

The results of antimicrobial activity of stem ethanol extracts of *W. obtusifolia* and *W. somnifera* were tested against microorganisms using agar well diffusion method are presented in table 1 and 2. Ethanol extract was active against both bacteria and fungi strains. In our study, in the species of *W. somnifera* a maximum inhibition zone was found to be 18mm in the concentration of 100µl, against an isolated human pathogenic bacteria *K.pneumoniae* and the moderate inhibition zone 17mm found in same 100µl

concentrations against *S.typhi*. The minimum inhibition zone 10mm was found in concentration of 10µl *E.coli.* The maximum inhibition zone from100µl was observed in *A.niger* with 20 mm. But in the case of *W. obtusifolia* in ethanol stem extract a maximum inhibition zone was found to be 28mm in the concentration of 100µl, against an isolated human pathogenic bacteria *K.pneumoniae* and the moderate inhibition zone 24mm found in same 100µl concentrations against *E.coli*. The minimum inhibition zone 12mm was found in concentration of 25µl *S.typhi.* In fungal pathogen a minimum inhibition zone was found in the concentration of 25µl was observed in *T.viride* with 10mm and maximum inhibition zone from100µl was observed in *A.niger* with 30mm. The present study was an enhanced activity found in *W. obtusifolia* when compared to *W. somnifera*. The former showed high degree of inhibition against *S.typhi* (11.1mm) whereas moderate antibacterial activity was associated with *K.pneumoniae* (8.0mm) and *Eschrichia coli* (7.2mm) [8,9,10,11,12,13,14,15,16].

Table 1
Stem ethanol extracts against bacterial and fungal isolates in W.somnifera

ORGANISM	Zone of inhibition (mm) at volume of stem sample loaded (µl)				
	25 µl	50 µl	75 µl	100 µl	Control
E.coli	10	12	15	18	13
Salmonella typhi	12	15	18	16	13
Klebsiella pneumoniae	12	14	18	17	15
A.niger	11	16	20	18	12
Trychoderma viride	10	13	15	17	12

Table 2
Stem ethanol extracts against bacterial and fungal isolates in W.obtusifolia

ORGANISM	Zone of inhibition (mm) at volume of stem sample loaded (µl)				
	25 µl	50 µl	75 µl	100 µl	Control
E.coli	12	15	20	24	15
Salmonella typhi	12	14	16	20	13
Klebsiella pneumoniae	15	20	24	28	13
A.niger	13	20	25	30	12
Trychoderma viride	10	12	14	16	12

The FT-IR and UV spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation[17]. The ethanol leaf extract of *Withania* species was passed into the FTIR, in *W. obtusifolia* the functional groups of the components were separated based on the peak area and the same was passed into UV spectroscopy for the electron transition of compounds. The results of FT-IR analysis was confirmed the presence of the secondary amine, cyclobutanes, hydroxyl compounds, amino acids, nitrite, trans and lower to cis, alkynes cyclopropanes, alkynes stretching which shows peaks range between 625.88 to 3443.60cm^{-1} but in the case of *W. somnifera* the functional groups of the components were separated based on its peak such as secondary amine, alkynes, hydroxyl compounds, tertiary amine, ketones, aldehyde, alkylamine and aliphatic primary amines stretching which shows peaks range between 666.99 to 3443.70cm^{-1} (Table 3 and Figure 1). The ethanol root extract of *W. obtusifolia* was passed into the FT-IR, the results of FT-IR analysis was confirmed the presence of the secondary amine, cyclobutanes, alkynes, hydroxyl compounds, diazoke tone, tertiary amide, ketones, amines, nitrate-aminoacids, for trans form stretching which shows peaks range between 619.13 to 3445.54cm^{-122} but in the case of *W. somnifera* the functional groups of the components were separated based on its peak such as secondary amines, ketones, sulphonic acid, aminoacids, nitroamina, amide i-band, sulphonic acid stretching which shows peaks range between 659.26 to 3438.69cm^{-122} were detected and this extracts was passed into the FTIR and UV radiation for the separation of the functional groups of the bioactive components based on its peak ratio and electron transition of compounds respectively (Table 4, 5 and Figure 2).

Table 3
FT-IR Peak value and its functional groups of leaf extracts of Withania Species

S.N.	Name of the plant species					
	W. obtusifolia (Leaf)			*W. somnifera* (Leaf)		
	Peak Area	Mode of vibration	Functional groups	Peak Area	Mode of vibration	Functional groups
1.	3443.60	N-H, Stretching	Secondary amine	3444.70	N-H, Stretching	Secondary amine
2.	2968.90	CH_2-asymmetric stretching	Cyclobutanes	2932.39	CH_2-asymmetric stretching	Alkynes
3.	2376.11	Conjugate chelation	Hydoxyl compounds	2521.07	Conjugate chelation	Hydoxyl compounds
4.	2074.44	Overtones and combination	Amino acids	2357.98	NH^+- Stretching	Tertiary amine salt
5.	1638.28	N-O, Stretching	Nitrite, Trans and lower to cis	2091.25	C=O, Stretching	Tertiary amide
6.	1412.70	CH_3-Bending vibration	Alkynes	1635.89	CH_2 –Bending	Ketones
7.	1018.31	Skeletal vibration	Cyclopropanes	1414.54	C-CHO, Skeletal	Aldehyde group
8.	625.88	CH-Bending vibration	Alkynes	1112.22	C-N, Stretching	Alkyl amine
9.	-	-	-	1021.72	O-N=O, Bending	For CIS form
10.	-	-	-	666.99	N-H, Wagging and Twisting	Aliphetic Primary amines

Table 4
FT-IR Peak value and its functional groups of root extracts of Withania Species

S.N.	Name of the plant species					
	W. obtusifolia (Root)			*W. somnifera* (Root)		
	Peak Area	Mode of vibration	Functional groups	Peak Area	Mode of vibration	Functional groups
1.	3445.54	N-H, Stretching	Secondary amine	3438.69	N-H, Stretching	Secondary amine
2.	2980.40	CH_2-asymmetric Stretching	Cyclobutanes	2967.53	C-N, Stretching	Ketones
3.	2929.78	CH_2-asymmetric Stretching	Alkynes	2359.47	O-H, Stretching	Sulphonic acid
4.	2728.71	CH_2, Stretching	Alkynes	2074.51	Overtones and combination	Amino acids
5.	2513.73	Polyvalent alcohols	Hydroxyl compounds	1638.95	NH_3^+ a symmetric bending	Amino acids
6.	2368.41	Conjugate chelation	Hydroxyl compounds	1458.80	N=O, Stretching	Nitrosamina
7.	2094.91	CO-CHN_2 Stretching	Diazoke tone	1410.23	C=S, Stretching	Amide I- band
8.	1634.28	C=O, Stretching	Tertiary amide	1018.10	SO_3, Stretching	Sulphonic acid
9.	1414.77	CH_2, Bending	Ketones	659.26	S-O, Stretching	Sulphonic acid
10.	1037.15	C-N, Stretching	Amines and their salts	-	-	-
11.	870.94	O-N, Stretching	Nitrate- aminoacids	-	-	-
12.	619.13	O-N=O, Bending	For Trans form	-	-	-

Figure 1
FT-IR Peak value and its functional groups of Withania Species

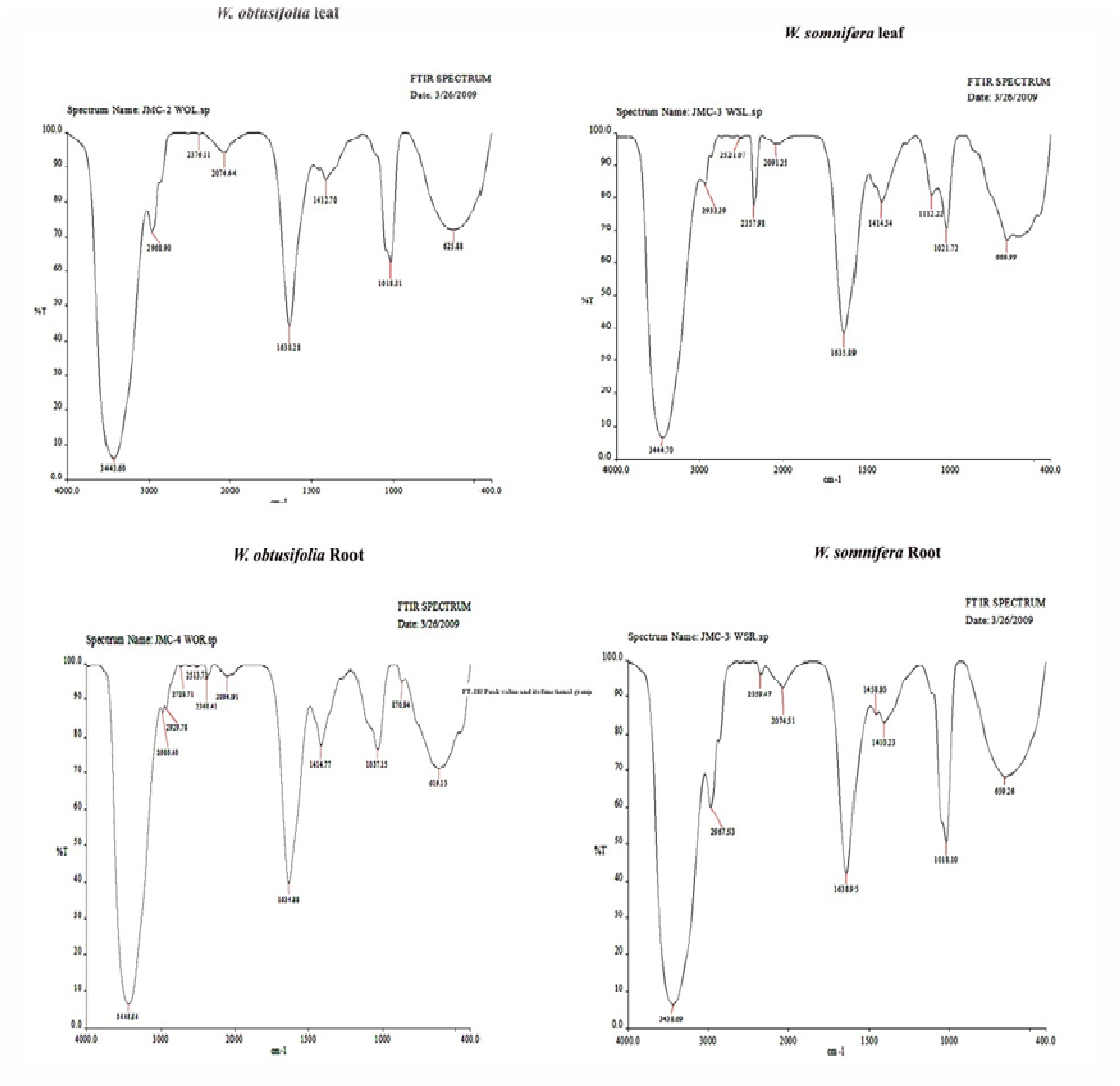
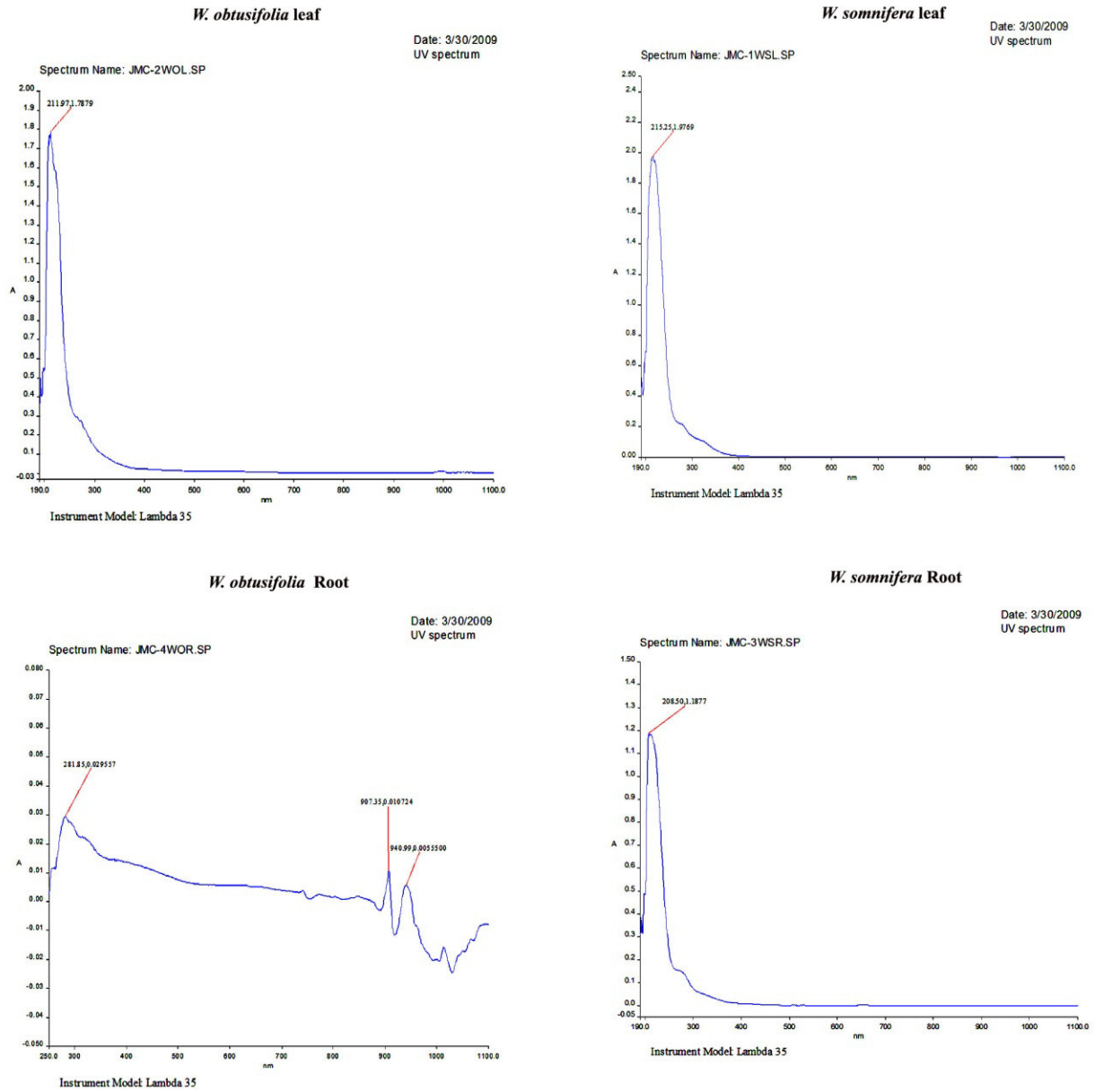


Table 5
UV-Spectrum peak value and its functional groups of leaf and root extracts of Withania Species

W. obtusifolia		***W. somnifera***	
Peak Value	**Functional Group**	**Peak value**	**Functional group**
211.977	n* transition to =OH group	215.25	n* transition to =OH group
281.85	Visible region	208.50	Visible region
907.35	Visible region	-	-
340.99	Visible region	-	-

Figure 2
UV Spectrum of Ethanol extract of Withania species



CONCLUSION

The *W. obtusifolia* and *W. somnifera* were usually misinterpreted because of their co-existence in mixed populations. The present investigation involves analysis of interspecific relationship between these two species. Specifically, this study was directed to the comparison of phytochemistry. In FT-IR analysis, the presence of the secondary amine, cyclobutanes, alkynes, hydroxyl compounds, diazoke tone, tertiary amide, ketones, amines, nitrate-aminoacids, for trans form stretching which shows peaks range between 619.13 to 3445.54cm^{-122} in *W. obtusifolia* but in the case of *W. somnifera* the functional groups of the components were separated based on its peak such as secondary amines, ketones, sulphonic acid, aminoacids, nitroamina, amide i-band, sulphonic acid stretching which shows peaks range between 659.26 to 3438.69cm^{-122} were detected.

REFERENCES

1. Wills JC, A dictionary of the flowering plants and ferns, Cambridge University Press. 1245, (1985).
2. Panneerselvan K., Jayapragasam M, Mani, DK and Bhavanisankar K., Indigenous knowledge of *Withania obtusifolia* in Trichy region, World Ayurveda summit in Bangalore, (2003).
3. Senthil Kumar M, Aslam A, Vinoth Kumar D, Ramachandran R and Shajahan A, "Comparative Studies on Leaf-Epidermal Features of *W. somnifera* and *W. obtusifolia* – Highly Medicinal Species of India", Advanced Biotech, 10 (02): 29-31 (2010).
4. Senthil Kumar M, Vinoth Kumar D, Ramachandran R and Shajahan A, "Anatomical Studies in *W. somnifera* L. (Dunal) - an Important Medicinal Plant of India" Advanced Biotech, 10 (01): 43-45, (2010).
5. Ramachandran R, Senthil Kumar and M,Vinoth Kumar D, Evaluation of Antimicrobial Activity of leaf extracts of *Wattakaka volubilis*, 4 (1): 6-10, (2014).
6. Karthishwaran K., Mirunalini S, Dhamodharan G, Krishnaveni M and Arulmozhi., Phytochemical investigation of ethanol extract of the leaves of Pergularia daemia, Journal Biol Sci, 10:242-246, (2010).
7. Rachh PR, Rachh MR, Soniwala MM, Joshi VD and Suthar AP, Effect of *Gymnema* Leaf extract on UV-Induced Damage on *Salmonella typhi*, Asian J Biol.Sci, 3:28-33, (2010).
8. Muhammad HS and Muhammmad S, The use of *Lawsonia inermis* (*henna*) in the management of burn wound infections, Afr. J. Biotech, 4: 934-937, (2005).
9. Desta B, Ethipian traditional herb drugs, Part II Antimicrobial activity of 63 medicinal plants, Journal of Ethnopharmacology, 39: 129-139, (1993).
10. Irobi ON, Anderson WH and Daramola SO, Antimicrobial activity of bark extracts of *Bridelia ferruginea* (Euphorbiaceae), Journal of Ethnopharmacology, 43: 185-190, (1994).
11. Vikasdhingra, Srinivasrao, Pakki and Lakshmi N, Antimicrobial activity of artemisinin and its precursors, Curr. Sci, 78: 709-713, (1999).
12. Gould D and Booker C, Applied Microbiology for Nurses, Aardvark Editorial Mendham, Suffolk, 75-94, (2000).
13. Madamome I and Afolayan A, Evaluation of antimicrobial activity of extracts from South African *Usnea barbata,* Pharmaceut, Biol, 41: 199-202, (2003).
14. Afolayan AJ, Extracts from the shoots of *Arctotis arctotoides* inhibit the growth of bacteria and fungi, Pharmaceutical Biology, 41 (1) 22-25, (2003).
15. Purohit MG, Shathaveerapa BK and Swamy HKS, Antimicrobial activity of various extracts of *Evolvulus alsinoides,* Indian Journal of Microbiology, 33: 77-78, (1995).
16. Amphawan N and Duang, Antifungal activity of turmeric oil extracts from *Curcuma longa,* Journal of Ethnopharmacology, 49: 163-169, (1995).
17. Aida P, Vila R, Freixa B, Adzet T and Conigueral S, Antifungal activity of Paraguyan plants used in traditional medicine, Journal of Ethnopharmacology 76: 93-98, (2001).
18. Natarajan, D. Yuvarajan, R., R. Srinivasan, Antibacterial potential and FT-IR analysis of *Gymnema kollimalayanum*, *International journal of Pharmaceutical Sciences Review and Research* 7 (2):167-169, (2013).