

Evaluation of herb-drug interaction of *Withania somnifera* with anti-rheumatic drugs through high-throughput screening

Satyajyoti KANJILAL¹ * , Ashok Kumar GUPTA² , Ranjana PATNAIK¹ , Amitabha DEY³ ,
Satyabrata MOHAPATRA⁴ 

¹ Department of Clinical Research, School of Biosciences & Biomedical Engineering, Galgotias University, Greater Noida, Uttar Pradesh, India.

² School of Medical and Allied Sciences, Galgotias University, Greater Noida, Uttar Pradesh, India

³ Bioassay Laboratory, Healthcare Division, Research and Development Centre, Emami Limited, Kolkata, India

⁴ Phytochemistry Laboratory, Healthcare Division, Research and Development Centre, Emami Limited, Kolkata, India

* Corresponding Author. E-mail: ksatyajyoti@gmail.com (S.K.); Tel. +91-09811868420.

Received: 07 August 2021 / Revised: 18 February 2022 / Accepted: 19 February 2022

ABSTRACT: Rheumatoid Arthritis is an autoimmune inflammatory joint disease. Disease-modifying anti-rheumatic drugs and Non-steroidal anti-inflammatory drugs are being used for its management and found to have better improvement when combined with a traditional medicine. *Withania somnifera* (Ashwagandha) is one among the medicinally important herb which is known to be beneficial in these conditions. The root of the plant is safely being used however, very less is known about the aerial part usage; and their interactions with anti-rheumatic drugs. Cytochrome P450 enzymes inhibition assay for subtypes CYP3A4 and CYP2D6 isozyme was used in concoction of hydroalcoholic and methanolic extracts of root and aerial part of the plant with the anti-arthritis drugs. All the four *Withania somnifera* extracts showed significantly higher ($P < 0.001$) IC₅₀ value against CYP3A4 and CYP2D6 (IC₅₀ > 100 µg/mL) compared to positive control ketoconazole and Quinidine respectively. Combination of formulation and anti-rheumatic drugs showed significantly ($P < 0.001$) less inhibition on CYP3A4 and CYP2D6 as compared to positive control. To conclude, methanolic and hydroalcoholic extracts of both aerial and root part of *Withania somnifera* mixture with anti-rheumatic drug regimen had negligible CYP3A4 and CYP2D6 inhibition.

KEYWORDS: *Withania somnifera*; Ashwagandha; Herb-drug interaction; High-throughput screening; CYP inhibition.

1. INTRODUCTION

Rheumatoid Arthritis is a progressive autoimmune inflammatory condition affecting the joints and associated tissues, which in due course affects the quality of life [1]. Management of the disease includes immunosuppression with conventional disease-modifying anti-rheumatic drugs (DMARDs i.e. hydroxychloroquine, methotrexate, leflunomide and sulphasalazine), inhibition of specific inflammatory process by Biologics, anti-inflammatory drugs (Non-steroidal anti-inflammatory drugs - NSAIDs) and corticosteroids [2, 3]. With the progression of the disease, patients are routinely being managed in a stepwise manner [4] and the combination DMARD therapy were found to be more effective than monotherapy [5]. The benefits of DMARDs also comes with adverse effects in prolonged administration [3] resulting in increased preference towards the use of Complementary and Alternative medicine (CAM) because of the holistic approach to health care [6]. These are often used as single or in various combinations and either as first line of therapy or in collaboration with other therapies [7, 8]. Methotrexate the most frequently used among the DMARDs [2] was found to have better improvement when combined with a traditional medicine in suboptimal relief cases of rheumatoid arthritis [9].

How to cite this article: Kanjilal S, Gupta AK, Patnaik R, Dey A, Mohapatra S. Evaluation of herb-drug interaction of *Withania somnifera* with anti-rheumatic drugs through high-throughput screening. J Res Pharm. 2022; 26(2): 334-344.

The herbs known for their Rasayana (rejuvenation) properties are hypothesised to improve nonspecific immune response to withstand antigenic challenge. They are considered to have adaptogenic and immunomodulatory properties [10, 11]. Large number of herbs in this category are well documented for their role in management of arthritis [12]. *Withania somnifera* (Aswagandha), a small evergreen shrub is one among them which is known to have therapeutic usage in inflammatory conditions [13]. The herbs are considered as safe in India by virtue of their usage since thousands of years in Indian system of medicine and folklore [14]. However, the therapeutic use of herbs warrants trained experts, evidence-based researches and complete safety profile understanding which otherwise can be hazardous to the humankind [15]. To authenticate safe use of a product in humans during its developmental stage, safety evaluation is an essential task [16]. The question arises regarding the safety of a particular herb when added in the prescription regimen [17]. The likelihood of herb-drug interactions is higher than drug-drug interactions because of multiple bioactive phyto-compounds which requires mapping with the prescription drugs [17, 18]. This leads to an evidence-based approach through various experimental and clinical models especially cytochrome P450 (CYP450) enzymes [19]. These enzymes in humans are involved in the metabolism of majority of drugs with approximately 50% through CYP3A4 and 25% by CYP2D6 [20, 21]. Inhibition of these enzymes may affect the metabolism of drugs when co-administered with herbs and can result in unexpected undesired effects leading to toxicity or can lead to diminished activity [22]. CYP inhibition studies have been conducted on ashwagandha root extracts with both CYP3A4 and CYP2D6 enzymes [23]. Primarily the roots of *Withania somnifera* (*W. somnifera*) are being used for its therapeutic use in form of whole root powder or extracts [24]. Roots possess anti-inflammatory property and have been extensively researched for its anti-arthritis potential [25, 26, 27]. Traditionally it is used in Indian system of medicine as anti-inflammatory and rejuvenator herb [28, 29]. However, few references are available for its use of other aerial parts [30, 31], especially the leaves having anti-inflammatory property and are being studied in treatment of arthritis [27, 32]. Fresh leaves are referred in ancient medicinal text to be used topically in rheumatoid arthritis [28]. The root part is safely being used since ages with some reference on aerial part however, very less is known about their interactions with anti-rheumatic drugs [33]. The present study is an attempt to understand the herb-drug interactions (HDI) of both the roots and the aerial part in their methanolic and hydroalcoholic extract forms when given in combination of the commonly used treatment of arthritis.

2. RESULTS AND DISCUSSION

2.1. Phyto-chemical analysis of WSEs

The phytochemical analysis of *W. somnifera* have revealed several groups of bioactive compounds such as flavonoids, tannin, alkaloids, steroidal lactones, and alkaloids [34]. The most therapeutically important chemicals are withanolides [35]. The present phytochemical study analysis also showed accumulation of withaferin A and withanolide A with variations in their concentrations in the different parts of Ashwagandha. The phyto-constituents Withaferin A and Withanolides are studied scientifically to possess immunomodulatory as well as anti-inflammatory properties thus making the herb a potential candidate in management of arthritis [36, 37].

The HPTLC analysis method was validated by ICH guidelines (International Conference on Harmonization). Concentrations of the compounds in sample chromatograms (Figure 1) were determined from the intensity of absorbance at 232 nm. Evaluation was made via peak areas versus withanolides amount in linear regression. The withanolides present in the extracts were identified by comparing HPTLC spectrum of the standards (Figure 2).

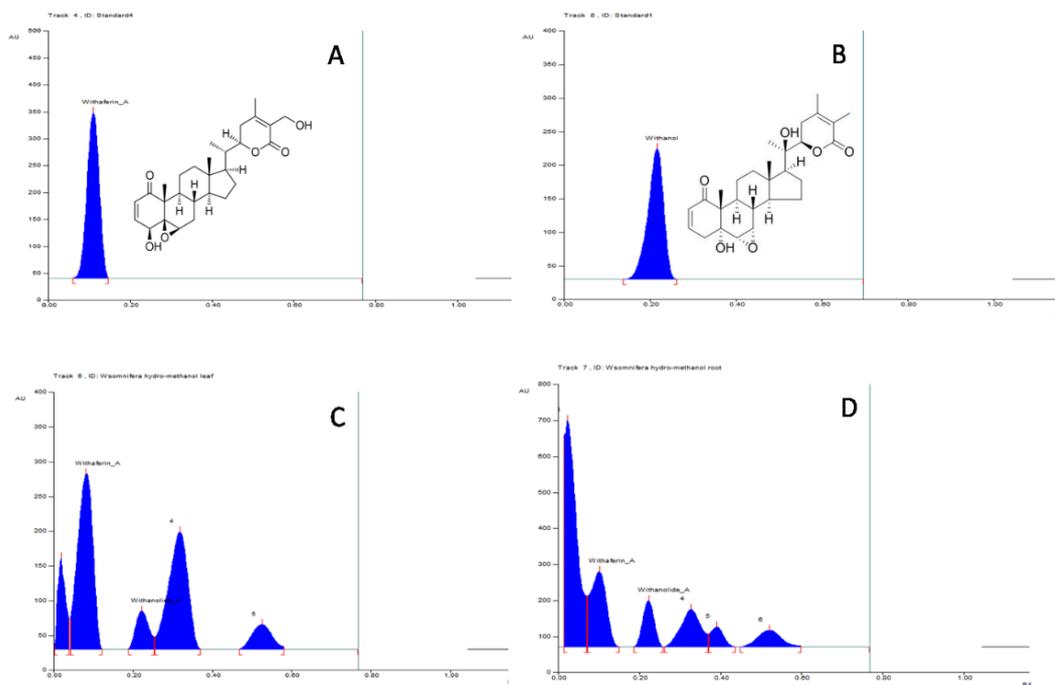


Figure 1. HPTLC chromatogram of standards and extracts
A: Withaferin A; B: Withanolide A; C: *W. somnifera* aerial – aqueous methanol extract;
D: *W. somnifera* root – aqueous methanol extract

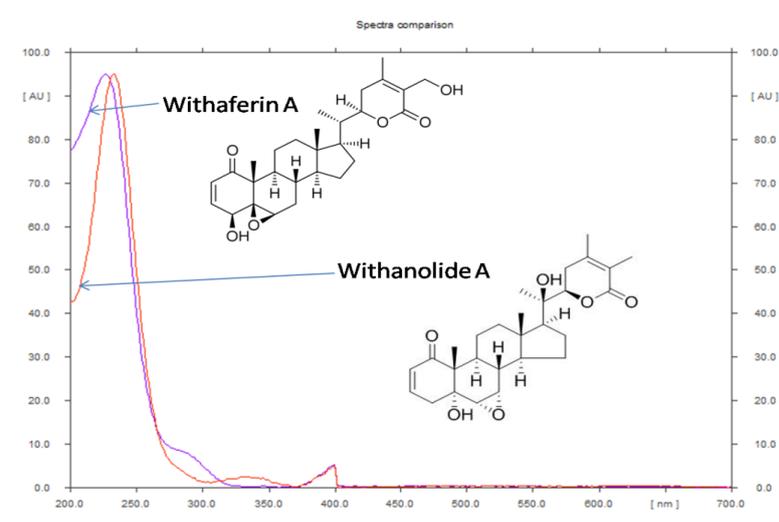


Figure 2. HPTLC spectrum of Withanolides

Estimation: The quantities of withanolides are present more in 50% aq-methanolic extracts of root part of *W. somnifera* (Table 1).

Table 1. Accumulation of withaferin A and withanolide A in various *W.somnifera* parts

S No	Plant parts	Withaferin A	Withanolide A
1	Aerial	0.70%	0.03%
2	Root	0.50%	0.08%

2.2. Herb-drug interactions of *W. somnifera*

For the CYP 3A4 assay, to authorize the correctness of the assays Ketoconazole was used as positive control and for accuracy samples were analyzed in triplicate. The percentage inhibition of the standard inhibitors was recorded in different concentrations and IC₅₀ values were calculated. IC₅₀ value for Ketoconazole was calculated to be 4.38 µg/ml (Table 2).

Table 2. Percentage inhibition of the standard inhibitors

Standard inhibitor [Concentration (µg/ml)]	Ketoconazole (CYP3A4 enzyme % inhibition)	Quinidine (CYP2D6 enzyme % inhibition)
10	100	100.00
5	64.39	75.17
2.5	31.55	53.38
1.25	15.75	30.44

The standard drugs of Methotrexate, Sulfasalazine, Hydroxychloroquine, Leflunomide, Prednisolone and Diclofenac were used in concentrations from 62.5, 125, 250 and 500 µg/ml. The percentage inhibition was found to be higher with IC₅₀ value being >500µg/ml (Figure 3 A). The *W. somnifera* extracts (WSEs) were used in concentrations of 12.5, 25, 50 and 100 µg/ml. The IC₅₀ values for all the four WSEs were found to be >100 µg/ml (Figure 3 B). The mixture of the standard drugs and WSEs were evaluated in concentration between 12.5 to 100 µg/ml. The percentage of inhibition showed similar trend as of individuals and the IC₅₀ was calculated to be >100µg/ml (Figure 3 C - F). For the CYP 2D6 assay, the positive control Quinidine was used and the IC₅₀ value was calculated as 2.70 µg/ml (Table 2). The assay results were similar to that of CYP3A4 in all the groups of the standard drug regimen, WSEs and combinations (Figure 4 A - F). The IC₅₀ values for standards were >500µg/ml, WSEs and combinations were >100 µg/ml. All the four WSE showed significantly higher ($P<0.001$) IC₅₀ value against CYP3A4 and CYP2D6 (IC₅₀ >100µg/mL) compared to positive control ketoconazole and Quinidine, respectively. Combination of formulation and anti-rheumatic drugs showed significantly ($P<0.001$) less inhibition on CYP3A4 and CYP2D6 as compared to positive control. Dose response inhibition studies showed that inhibition of CYP3A4 and CYP2D6 by WSE from both the roots and aerial part were concentration dependent (Figure 3 and 4).

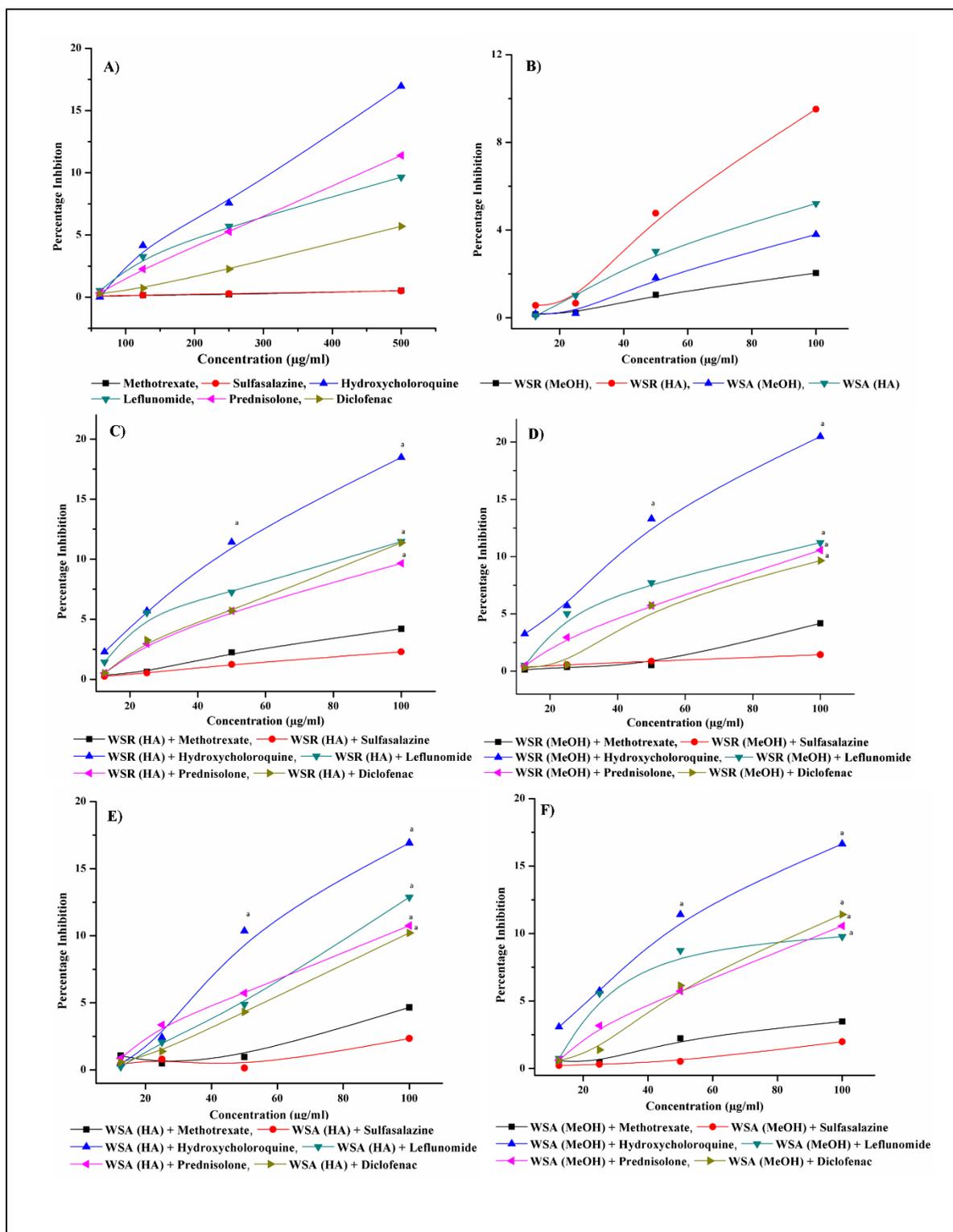


Figure 3. Herb drug interaction of *Withania somnifera* and Anti-rheumatic drugs on CYP 3A4 inhibition assay. Percentage inhibition of: A: Anti-rheumatic drugs; B: Extracts of *W. somnifera* root and aerial parts; C: Cocktail of WSR (HA) and Anti-rheumatic drugs; D: Cocktail of WSR (MeOH) and Anti-rheumatic drugs; E: Cocktail of WSA (HA) and Anti-rheumatic drugs; F: Cocktail of WSA (MeOH) and Anti-rheumatic drugs; Statistically significant difference ($p < 0.001$) were denoted with the symbol "a" as compared to standards.

Anti-rheumatic drugs: Disease-modifying antirheumatic drugs (DMARDs) and Anti-inflammatory drugs; WSR (HA): *W. somnifera* root – aqueous alcoholic extract; WSR (MeOH): *W. somnifera* root – aqueous methanol extract; WSA (HA): *W. somnifera* aerial – aqueous alcoholic extract; WSA (MeOH): *W. somnifera* aerial – aqueous methanol extract.

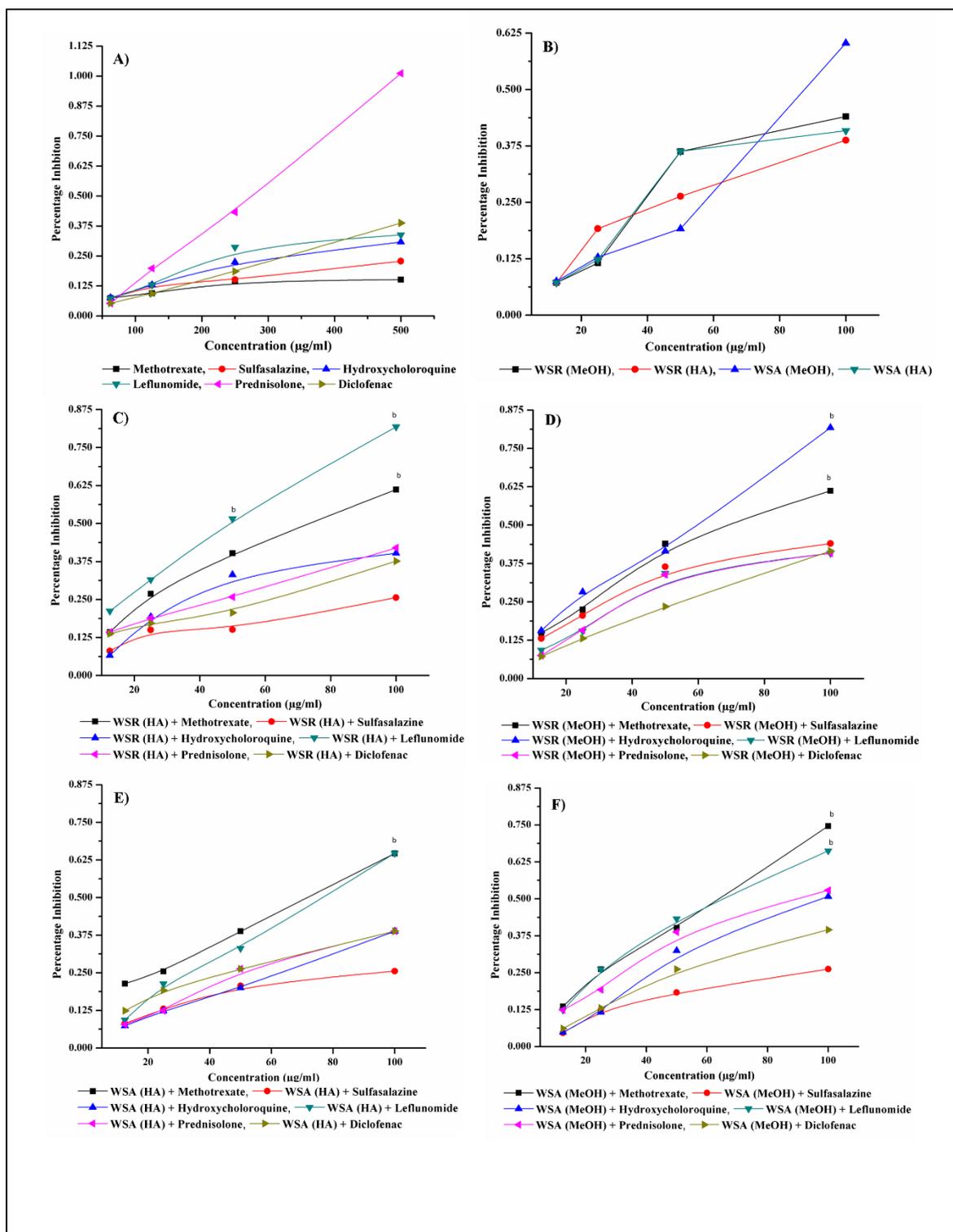


Figure 4. Herb drug interaction of *Withania somnifera* and Anti-rheumatic drugs on CYP 2D6 inhibition assay. Percentage inhibition of: A: Anti-rheumatic drugs; B: Extracts of *W. somnifera* root and aerial parts; C: Cocktail of WSR (HA) and Anti-rheumatic drugs; D: Cocktail of WSR (MeOH) and Anti-rheumatic drugs; E: Cocktail of WSA (HA) and Anti-rheumatic drugs; F: Cocktail of WSA (MeOH) and Anti-rheumatic drugs; Statistically significant difference ($p < 0.001$) were denoted with the symbol “b” as compared to standards.

Anti-rheumatic drugs: Disease-modifying antirheumatic drugs (DMARDs) and Anti-inflammatory drugs; WSR (HA): *W. somnifera* root – aqueous alcoholic extract; WSR (MeOH): *W. somnifera* root – aqueous methanol extract; WSA (HA): *W. somnifera* aerial – aqueous alcoholic extract; WSA (MeOH): *W. somnifera* aerial – aqueous methanol extract

The collaborative therapy may have synergistic or negative effects. *W. somnifera* in a collaborative therapy with anti-tubercular drugs such as rifampicin, pyrazinamide, ethambutol and isoniazid did show encouraging results [8]. It is used in combination therapy for musculoskeletal painful conditions [38]. As a precaution, results in other studies are suggestive that it should be thoughtfully used in Parkinson's disease, may augment paclitaxel effects [39] and potentiate the sedative effects of barbiturates and some benzodiazepines [40].

Despite of its usage only few evidences are available of HDI on the root extracts but not on the aerial part of the plant. Savai et.al. documented no inhibition of CYP3A4 and CYP2D6 with root extracts of *W. somnifera* and isolated phyto-constituents withaferin-A, withanolide-A and withanoside-IV [41, 42]. However, in an in-vivo experiment on methanolic extract of *W. somnifera* (500 mg/kg) in rats, it induced CYP1A enzyme activity and altered phenacetin pharmacokinetic profile [42]. The present study also deciphered the no interaction potential of *W. somnifera* standardized methanolic and hydro-methanolic extracts from both the aerial and root with CYP3A4 and CYP2D6 when tested with anti-arthritis drug regimen through specific high-throughput screening assays.

3. CONCLUSION

Methanolic and hydroalcoholic extracts obtained from both aerial and root part of the plant *Withania somnifera* mixture with anti-rheumatic drug regimen had negligible CYP3A4 and CYP2D6 inhibition. Thus, co-administration of formulation prepared with these extracts along with selective disease modifying anti-rheumatic drugs may be considered as safe without any significant CYP inhibition mediated herb-drug interaction. However, there remains always scope for further research to get a comprehensive profile of the herb such as irreversible inhibition mechanism, phase II conjugating enzyme activity and Clinical studies.

4. MATERIALS AND METHODS

4.1. Chemicals and Reagents

Vivid™ CYP3A4 Blue Screening Kit (catalogue number P2856), Vivid® CYP2D6 Blue (Cat. no. P2972), Quinidine and ketoconazole were procured from Sigma Chemical Co. (St. Louis, MO, USA). All the solvents and chemicals for standardization and sample preparation i.e. n-hexane (AR grade), ethyl acetate (AR grade), n-butanol (AR grade), and methanol (HPLC grade) were purchased from Merck & Co., USA.

4.2. Raw materials and DMARDS

W. somnifera raw material of aerial part Lot No.: ASHW/PLPLR30/NOV19 and Ashwagandha aerial part hydroalcoholic extract 5%, Lot No: ASHW/PLPL30/NOV were procured from Phyto Life sciences Pvt Ltd, Ahmedabad, Gujarat. The root powder was obtained from Emami Ltd, Research and Development Centre, Kolkata. Both the raw material of aerial and root were further used to get the methanolic and hydroalcoholic extracts in the phytochemistry laboratory of Emami Research and Development Centre. The Anti-arthritis drugs were purchased from the local pharmacist - Methotrexate 25 mg (Folitrax) Batch No. At 071110, Hydroxychloroquine (HCQS 200) Batch No. GPD089010BH, Leflunomide (Lefna 20) Batch No. GOVO19005AS, Sulfasalazine (Saaz) Batch No. ECL079007AS marketed by IPCA Laboratories Ltd, Mumbai. Prednisolone (Wysolone 10) Batch No. 75349S of Pfizer India and Diclofenac (Voveran 50) Batch No. 195009MB of Novartis India Ltd were also used.

4.3. Preparation of *W. somnifera* extracts (WSEs)

100 g of dried powdered material of roots and aerial part of *W. somnifera* were defatted with petroleum ether (AR grade) at room temperature for 18 hours with intermittent shaking. This was followed by extraction (cold maceration technique) using 500 ml of methanol, aqueous-methanol (water and methanol in equal ratio) and water individually to check the yield of methanolic, aqueous-methanolic, and aqueous extracts of both plants. Alcoholic and hydroalcoholic extracts were evaporated to dryness using rotary vacuum evaporator at 50 °C, whereas aqueous extracts were dried by lyophilization. Further, all the extracts were dried and stored in vacuum desiccator till use. Initial HPTLC qualitative studies showed that the aqueous-methanolic extracts of both plant parts contain higher amounts of withanolides than all other extracts. Further a HPTLC quantitative analysis has been done on aqueous-methanol extracts of both the plant parts with respect to two withanolide markers (withaferin A and withanolide A) [27, 43].

4.4. HPTLC instrumentation and chromatographic conditions

4.4.1. Sample preparation

Accurately weighed 1.0 g of leaf and root powder was soaked separately in 50 ml conical flask with 20 ml of 50% aq-methanol. Both the samples were sonicated at 60 °C for 30 minutes. The samples were filtered through Whatmann No.1 filter paper to 25 ml volumetric flasks. Then the volumetric flasks are made up to 25 ml volume. The concentration of sample solution was prepared as 40 mg/ml.

Accurately weighed 10.0 mg of both the standards (withaferin A and withanolide A) were dissolved with methanol in 5 ml volumetric flasks. The flasks were made up to the volume to 5.0 ml. Stock solution concentration was 2.0 mg/ml. Working standard solution were prepared by further 10-fold dilution of the stock solutions. Visualization of the TLC plate is being done at 254 nm and after derivatization with a freshly prepared anisaldehyde spray reagent.

4.4.2. HPTLC instrumentation

The chromatographic estimation was performed by using silica gel aluminum sheet plate 60 F254 (E. Merck, Darmstadt, Germany). The samples, 10µL each were applied on the plate by using Linomat V sample applicator. Plates were developed using a mobile phase consisting of n-hexane: ethyl acetate (3:1, v/v) in a twin-trough glass chamber. The length of chromatogram run was 6.0 cm. The slit dimension settings of length 5.0 mm and width 0.45 mm, and a scanning rate of 20 mm s⁻¹ were employed.

Densitometric scanning was performed using a CAMAG TLC Scanner 4 in the absorbance mode at 232 nm and operated by winCATS planar chromatography version 1.4.9.

4.5. CYP3A4 and CYP2D6 isozyme inhibition assay

The inhibition assays were conducted by adapting the method narrated by Li et.al. [44], and as per the standard protocol provided with Vivid™ CYP3A4 and CYP2D6 Blue Screening Kit (Invitrogen). These Screening Kits allow rapid measurement of the drug and Cytochrome P450 enzymes interactions by using a “mix-and-read” fluorescent assay technique. These are specifically designed for high-throughput screening in multiple-well plates. Separately, each 40 µl of positive inhibition control (Ketoconazole for 3A4 and Quinidine for 2D6) and/or test samples i.e. WSEs, Cocktails of WSEs and Anti-arthritic drugs, and Drugs were added in Black 96 well plate. 40 µl of buffer solution was added as solvent control. 50 µl CYP450 BACULOSOMES® Plus (CYP enzyme) and 100 µl Vivid® Regeneration System was mixed to prepare the Master Pre-Mix (10 nM), and 50 µl of this Master Pre-Mix was dispensed to each well. The 96-well plates were kept for 10 minutes of incubation at room temperature to allow the compounds to interact with the CYP3A4 and CYP2D6. The reaction was started by adding 10 µl per well of the Vivid® Substrate (BOMR) and NADP+(2 mM BOMR, 1000 µM NADP+). Further, the plates were incubated for 1 hour for 37°C. In order to extinguish the reaction finally, the stop reagent was added to each well. Fluorescent microplate reader (SpectraMax i3x from Molecular Devices, USA) was used to measure the fluorescence intensity with excitation and emission wavelength of 535 and 590 nm respectively. Percentage of inhibitions and their corresponding IC50 values were calculated basis the formula described in Pandit et al., 2017 [22]. The percent inhibition was calculated due to presence of test compound or positive inhibition using the below mentioned equation:

$$\% \text{ of Inhibition} = [1 - (X - B) / (A - B)]$$

Where X denotes the fluorescence intensity observed in the presence of test compound, A is the fluorescence intensity observed without inhibitor (solvent control or no inhibitor control, as appropriate), and B is the fluorescence intensity observed with the positive inhibitor control.

4.6. Statistical analysis

The results were analyzed using one-way analysis of variance (ANOVA), followed by Tukey post-hoc test. Data are expressed as mean ± standard deviation (S.D) and the differences between groups were considered to be statistically significant when p < 0.05. The statistical analysis was performed in GraphPad Prism® 5.0 software (CA, USA), using the four-parameter logistic non-linear regression model. Graphs are made using OriginPro8 software.

Acknowledgements: The authors would like to express their gratitude to Emami Limited, Kolkata, India for providing support for this work. The authors always remain thankful to the department of Pharmacy, and Clinical research of

Galgotias university, Uttar pradesh, India for necessary guidance to complete the work. The authors also are thankful to all the authors and publishers where from the selected contents have been referred for this work.

Author contributions: Concept – S.K., A.G., R.P., A.D.; Design – S.K., A.D., A.G., R.P.; Supervision – A.D., S.M., A.G., R.P.; Resources – A.D., S.M., A.G., R.P.; Materials – S.K., A.D., S.M.; Data Collection and/or Processing – S.K., A.D., S.M.; Analysis and/or Interpretation – S.K., A.D., S.M., A.G.; Literature Search – S.K.; Writing – S.K., A.D., S.M.; Critical Reviews – S.K., A.G., R.P., A.D., S.M.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

- [1] Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ, Xu J. Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. *Bone Res.* 2018;6:15. [\[CrossRef\]](#)
- [2] Singh JA, Furst DE, Bharat A, Curtis JR, Kavanaugh AF, Kremer JM. Update of the 2008 American College of Rheumatology recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treatment of rheumatoid arthritis. *Arthritis Care Res* 2012;64:625-639 [\[CrossRef\]](#)
- [3] Gullick NJ, Scott DL. Co-morbidities in established rheumatoid arthritis. *Best Pract Res Clin Rheumatol.* 2011;25:469-483. [\[CrossRef\]](#)
- [4] Breedveld FC, Combe B. Understanding emerging treatment paradigms in rheumatoid arthritis. *Arthritis Res Ther.* 2011;13:S3. [\[CrossRef\]](#)
- [5] Calgüneri M, Pay S, Calışkaner Z, Apraş S, Kiraz S, Ertenli I, Cobankara V. Combination therapy versus monotherapy for the treatment of patients with rheumatoid arthritis. *Clin Exp Rheumatol.* 1999;17:699-704.
- [6] Hasan SS, Ahmed SI, Bukhari NI, Loon WC. Use of complementary and alternative medicine among patients with chronic diseases at outpatient clinics. *Complement Ther Clin Pract.* 2009;15:152-157. [\[CrossRef\]](#)
- [7] Ayurvedic Standard Treatment Guidelines. 1st Ed. 2017. Ministry of AYUSH Government of India, New Delhi, India. <https://main.ayush.gov.in/sites/default/files/ASTG%20Book%20%282%29.pdf>. (Accessed: 20 July 2020).
- [8] Debnath PK, Chattopadhyay J, Mitra A, Adhikari A, Alam MS, Bandopadhyay SK, Hazra J. Adjunct therapy of Ayurvedic medicine with anti-tubercular drugs on the therapeutic management of pulmonary tuberculosis. *J Ayurveda Integr Med.* 2012;3:141-149. [\[CrossRef\]](#)
- [9] Kogure T, Tatsumi T, Sato H, Oku Y, Kishi D, Ito T. Traditional herbal medicines (Kampo) for patients with rheumatoid arthritis receiving concomitant methotrexate: a preliminary study. *Altern Ther Health Med.* 2010;16:46-51.
- [10] Chopra A, Doiphode VV. Ayurvedic medicine. Core concept, therapeutic principles, and current relevance. *Med Clin North Am.* 2002;86(1):75-89. [\[CrossRef\]](#)
- [11] Kumar D, Arya V, Kaur R, Bhat ZA, Gupta V K, Kumar V. A review of immunomodulators in the Indian traditional health care system. *J Microbiol Immunol Infect.* 2012;45(3):165-184. [\[CrossRef\]](#)
- [12] Chopra, A. Ayurvedic Medicine and Arthritis. *Rheum Dis Clin North Am.* 2000; 26:133-144. [\[CrossRef\]](#)
- [13] Gupta A, Katiyar CK, Sastry JLN, Kanjilal S. Ayurvedic Pharmacopoeia of India: A Ready Reckoner. In: Katiyar CK, Khare CP, ed. *Ayurveda at the turning point*, 1st ed., Kruger Brentt, United Kingdom, 2017, pp.351-579.
- [14] Sharma GK. Medicinal plants folklore and Ayurvedic system of medicine in the Indo-Tibetan outer Himalayas. *J Tenn Acad Sci.* 2000;75:38-41.
- [15] Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol.* 2014;4:177. [\[CrossRef\]](#)
- [16] Atuah KN, Hughes D, Pirmohamed M. Clinical pharmacology: special safety considerations in drug development and pharmacovigilance. *Drug Saf.* 2004;27:535- 554. [\[CrossRef\]](#)
- [17] Williamson EM. Drug Interactions Between Herbal and Prescription Medicines. *Drug Saf* 2003;26:1075-1092. [\[CrossRef\]](#)
- [18] Izzo AA. Herb-drug interactions: an overview of the clinical evidence. *Fundam Clin Pharmacol.* 2005;19:1-16. [\[CrossRef\]](#)
- [19] Coxeter PD, McLachlan AJ, Duke CC, Roufogalis BD. Herb-drug interactions: an evidence based approach. *Curr Med Chem.* 2004;11:1513-1525. [\[CrossRef\]](#)

- [20] Tang W, Stearns RA. Heterotropic cooperativity of cytochrome P450 3A4 and potential drug-drug interactions. *Curr Drug Metab.* 2001;2:185-198. [\[CrossRef\]](#)
- [21] Wang B, Yang LP, Zhang XZ, Huang SQ, Bartlam M, Zhou SF. New insights into the structural characteristics and functional relevance of the human cytochrome P450 2D6 enzyme. *Drug Metab Rev.* 2009;41:573-643. [\[CrossRef\]](#)
- [22] Pandit S, Kanjilal S, Awasthi A, Chaudhary A, Banerjee D, Bhatt BN, Narwaria A, Singh R, Dutta K, Jaggi M, Singh AT, Sharma N, Katiyar CK. Evaluation of herb-drug interaction of a polyherbal Ayurvedic formulation through high throughput cytochrome P450 enzyme inhibition assay. *J Ethnopharmacol.* 2017;197:165-172. [\[CrossRef\]](#)
- [23] Savai J, Pandita N, Chintamaneni M. Investigation of CYP1A interaction potential of *Withania somnifera* in rat and human liver microsomes. *Int J Pharm Pharm Sci* 2015;7:273-278.
- [24] WHO monograph on selected medicinal plants, World Health Organization. *Radix Withaniae*. Available at: https://www.academia.edu/11956300/WHO_MONOGRAPH_ON_SELECTED_MEDICINAL_VOL_4. (Accessed on 25 July 2020).
- [25] Khan MA, Ahmed RS, Chandra N, Arora VK, Ali A. In vivo, Extract from *Withania somnifera* root ameliorates arthritis via regulation of key immune mediators of inflammation in experimental model of arthritis. *Antiinflamm Antiallergy Agents Med Chem.* 2019;18(1):55-70. [\[CrossRef\]](#)
- [26] Gupta A, Singh S. Evaluation of anti-inflammatory effect of *Withania somnifera* root on collagen-induced arthritis in rats. *Pharm Biol* 2014;52(3):308-320. [\[CrossRef\]](#)
- [27] Kanjilal S, Gupta AK, Patnaik RS, Dey A. Anti-inflammatory activity of aerial and root extracts of *Withania somnifera*: A comparative experimental evidence. *Int J Curr Res Rev* 2021;13(7):142-148. [\[CrossRef\]](#)
- [28] Ashwagandha. In: Mishra AB, Vaisya SR, ed. *Bhavaprakasa of Sri Bhavamishra Guduchyadivarga*, 8th Ed., Chaukhambha Sanskrit Bawan, Varanasi, India, 1993, pp.393-394.
- [29] Anonymous. *The Ayurvedic Pharmacopoeia of India. Part I. 1st ed. Vol. 1.* New Delhi: Government of India, Ministry of Health and Family Welfare, Department of AYUSH 2001; pp. 19-20
- [30] Nadkarni AK, Dr K M Nadkarni's *Indian Materia Medica*, Popular Parakashan, Bombay, 1976.
- [31] Kapoor LD, *Handbook of Ayurvedic Medicinal Plants*, CRC Press, London, 2001, pp. 337-338
- [32] Ramakanth GS, Uday Kumar C, Kishan PV, Usharani P. A randomized, double blind placebo controlled study of efficacy and tolerability of *Withania somnifera* extracts in knee joint pain. *J Ayurveda Integr Med.* 2016;7(3):151-157. [\[CrossRef\]](#)
- [33] Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha): a review. *Altern Med Rev.* 2000;5(4):334-346.
- [34] Kalra R, Kaushik N. *Withania somnifera* (Linn.) Dunal: a review of chemical and pharmacological diversity. *Phytochem Rev.* 2017;16, 953-87. [\[CrossRef\]](#)
- [35] Kumar V, Dey A, Hadimani MB, Marcović T, Emerald M. Chemistry and pharmacology of *Withania somnifera*: An update. *TANG.* 2015;5:1-3. [\[CrossRef\]](#)
- [36] Dar NJ, Hamid A, Ahmad M. Pharmacologic overview of *Withania somnifera*, the Indian Ginseng. *Cell Mol Life Sci* 2015;72(23):4445-60. [\[CrossRef\]](#)
- [37] Budhiraja RD, Sudhir S. Review of biological activity of withanolides. *J Sci Ind Res* 1987;46:488-491.
- [38] Tiwari R, Chakraborty S, Saminathan M, Dhama K, Singh SV. Ashwagandha (*Withania somnifera*): Role in safeguarding health, Immunomodulatory effects, combating infections and therapeutic applications: A review. *J Biol Sci.* 2014;14:77-94. [\[CrossRef\]](#)
- [39] Ulbricht C, Chao W, Costa D, Rusie-Seamon E, Weissner W, Woods J. Clinical Evidence of Herb-Drug Interactions: A Systematic Review by the Natural Standard Research Collaboration. *Curr Drug Metab.* 2008;9:1063-1120. [\[CrossRef\]](#)
- [40] Williamson EM. Interactions between herbal and conventional Medicines: the role of cytochrome P450 enzymes and p-glycoprotein. *Pharmacologyonline.* 2006;2:200-205.
- [41] Savai J, Varghese A, Pandita N. Lack of the cytochrome P450 3A interaction of methanolic extract of *Withania somnifera*, Withaferin A, Withanolide A and Withanoside IV. *J Pharm Negative Result.* 2013;4:26-32. [\[CrossRef\]](#)
- [42] Savai J, Varghese A, Pandita N, Chintamaneni M. Investigation of CYP3A4 and CYP2D6 interactions of *Withania somnifera* and *Centella asiatica* in human liver microsomes. *Phytother Res.* 2015;29:785-790. [\[CrossRef\]](#)

- [43] Ojha S, Raj A, Roy A, Roy S. Extraction of total phenolics, flavonoids and tannins from *paederia foetida* L. leaves and their relation with antioxidant activity. *Pharmacog J.* 2018;10(3):541-7. [[CrossRef](#)]
- [44] Li G, Simmler C, Chen L, Nikolic D, Chen SN, Pauli GF, van Breemen RB. Cytochrome P450 inhibition by three licorice species and fourteen licorice constituents. *Eur J Pharm Sci.* 2017;109:182-190. [[CrossRef](#)]

This is an open access article which is publicly available on our journal's website under Institutional Repository at <http://dspace.marmara.edu.tr>.