

CYP-450 Mediated Herb-Drug Interactions of Ashwagandha and AYUSH-64 Used in COVID-19 Integrative Management: A Case Example of Remdesivir

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Abstract

Aim

Withania somnifera Dunal (WS), known as *Ashwagandha* and AYUSH-64, a polyherbal formulation are repurposed for the management of COVID-19. The extensive use of these botanicals as home remedy along with other drugs for managing multifarious disease conditions is increasing over nations. This raises high chances of herb-drug interactions (HDIs) which may be beneficial, harmful, or even fatal. Therefore, current study aimed to explore the CYP mediated herb-drug interactions (HDIs) of *Ashwagandha* and AYUSH-64 along with case example of remdesivir to harness the best of these HDIs for integrative management of COVID-19

Materials and Methods

The aqueous extract of *Ashwagandha* and AYUSH-64 were characterized by LC-MS/MS. The *in-silico* pharmacokinetic (ADME) parameters were studied by using ADME tool. The *in-vitro* CYP-450 (CYP3A4, 2C8, 2D6) inhibition studies of WS and AYUSH-64 alone and in combination with a remdesivir were carried out resembling clinically scenario.

Results

Total of 11 and 24 phytoconstituents were identified from the aqueous extract of *Ashwagandha* and AYUSH-64. The *in-silico* ADME studies revealed that most of the phytoconstituents showed good oral bioavailability, drug likeliness, GI affinity and some of them displayed CYP-450 inhibitory activity. The *in-vitro* CYP-450 studies of remdesivir showed moderate inhibitory effect on CYP3A4, 2C8, 2D6. The aqueous extract of *Ashwagandha* did not show any inhibitory activity towards all the studied CYP's alone and in combination with remdesivir (IC₅₀ >100µg/ml). Whereas, AYUSH-64 also followed the same trend but showed moderate inhibitory effect on CYP2C8 (IC₅₀ <100µg/ml).

Conclusion

Ashwagandha did not exhibit HDIs with the CYP3A4, CYP2C8 and CYP2D6 thereby seem to be safe to co-administer with respective substrates. Whereas, AYUSH-64 only showed moderate HDIs towards CYP2C8 substrate among studied CYP enzymes. Caution is therefore warranted for prescribing AYUSH 64 along with CYP2C8 substrate drugs.

1. Introduction

Modern system of medicine has emerged as the primary choice for the treatment of nearly all types of health-related issues (1). On the other hand, the Traditional Complementary and Alternative Medicine (TCAM or CAM) like Ayurveda, Siddha, Unani, Sowa-Rigpa, Chinese medicine, etc. are gaining popularity due to their higher safety and long-term efficacy (2). The currently available treatment modalities of modern medicine as well as CAM are still struggling to handle the multi-factorial chronic disorders like cancer, diabetes, arthritis, COVID-19, etc. (1, 2). Therefore, there is need to find the way to use the strengths of both systems of medicines for betterment of health care sector. But, the patients of such chronic illnesses directly/indirectly take combinational/multimodal therapy with or without the knowledge of physicians, leading to potential herb-drug interactions (HDIs) (3).

The World Health Organization (WHO) data and literature shows that, more than 70-80% world's population uses CAM for their health care needs. Particularly in western countries CAM has become increasingly popular over the last few decades (1, 2). The health-seeking behaviour studies from various parts of the world suggests widespread use of TCAM in noncommunicable diseases (NCDs) (4–6). This pattern is similar in high-income countries as well as low and middle-income countries. Most of the countries do not have evidence-based policies nor regulations to rationale the concomitant utilization of therapies (3). This phenomenon needs systematic scientific investigations, especially for herb-drug interaction studies. This study will be important in the Indian scenario where most of the patients use herbs and drugs together. However, concomitant usage of herbs and conventional medicines globally could be much higher as healthcare professionals often do not ask about herbal remedies when prescribing and patients do not volunteer that they are taking them (2, 3). Indeed, such a scenario of concomitant usage of herbs/CAM and conventional medicines brings with it the potential problem of HDI's and this issue has emerged as a major hurdle/problem in our journey towards integrative medicine (IM) (7).

Integrative medicine refers to the blending of conventional and evidence-based complementary medicines and therapies with the aim of using the most appropriate of either or both modalities for efficient patient care (2, 7, 8). In short, IM utilizes all appropriate, evidence-based therapies to achieve health. For example, *Aswagandha*, have been widely accepted as a novel complementary therapy for integrative oncology care (9). *Aswagandha*, *AYUSH-64*, *Tinospora cordifolia*, *Piper longum*, etc. are being strongly exploited in the integrative management of COVID-19 (10–16). In addition, to that they are also being used as home remedies to combat the various communicable and non-communicable diseases (10–16). In addition to this, patients of such chronic illnesses directly/indirectly take multimodal therapy with or without the informing to the physicians, leading to potential HDIs. Such HDIs may be beneficial, harmful or even fatal (17).

HDIs may possibly observed, both at pharmacodynamic and pharmacokinetic (at any stage of ADME) level (18). The interactions at the site of metabolism are eminently noticed due to their complex metabolic processes (2). Most of the xenobiotics and herbal drugs are known to metabolise by phase I metabolising enzymes rather than Phase II. Of which, cytochrome P450 mediated phase I metabolising enzymes accounts for xenobiotic transformation of 90% of drugs and herbal medicines (19, 20). In order to anticipate the possible drug interactions at the metabolism site for better therapeutic and safety profiles of IM, it is always necessary to study the metabolic interactions of both allopathic and herbal drug candidates (21). Despite of all 60 CYP isoforms that are available in human genome, the six CYP enzymes namely 1A2, 2C8, 2C9, 2C19, 2D6 and 3A4 are majorly engaged in the metabolism of 70-90% of drugs (20).

Therefore, the present study focuses assessing the probable HDIs associate with the use of *Aswagandha* and *AYUSH-64* in the management of COVID-19. In this paper, we have performed the phytochemical characterization of *Aswagandha* and *AYUSH-64* by LC-MS/MS. The *in-silico* pharmacokinetic (ADME) parameters were explored for the identified phytoconstituents of both the herbal extracts. We have also used remdesivir (key antiviral, antiCOVID-19 drug) as a representative example for HDI studies. As the remdesivir gets metabolised by CYP3A4, 2C8, and 2D6 so, the studies on the above listed selected CYP enzymes have a profound importance to predict clinical HDIs for effective and safe management of the disease due to probable HDIs.

2. Material And Methods

2.1 Chemicals reagents and solvents

Testosterone, ketoconazole, 6 β -hydroxy testosterone, paclitaxel, rosiglitazone, remdesivir, NADPH, 6-hydroxy paclitaxel, dextromethorphan HBr, dextropran, quinidine, NADPH, Mixed gender HLM (Human liver microsomes: Pool of 50) were received from Sigma Aldrich. Pvt. Ltd, India. Water, methanol, acetonitrile of LC-MS grade was procured from Merck life science, Pvt. Ltd, India. The aqueous extracts of *Withania somnifera* and *AYUSH-64* were received from Central Council for Research in Ayurvedic Sciences (CCRAS), Ministry of AYUSH, Govt. of India. The aqueous extract of *Aswagandha* where prepared as per the Ayurvedic procedure. Whereas the *AYUSH 64* is a polyhedral formulation prepared as per Ayurvedic logic which established marketed product of CCRAS as an Ayurvedic Proprietary Medicine.

2.2. Phytochemical characterization of *Aswagandha* and *AYUSH-64* by using LC-MS/MS

The lyophilized aqueous extracts of *Aswagandha* and *AYUSH-64* was reconstituted at a concentration of 10 mg/mL in 100% methanol followed by sonication of about 30 min. The sample was further filtered by using 0.2 mm filter and LC-MS/MS was used to characterize the extract. The HSS-T3 C18 column (2.1x 100mm, 1.8 μ m, 100 \AA ; Waters Corporation) were used in "UHPLC Ultimate 3000" and the column oven temperature was maintained at 40°C. Mobile phase A and B contains water with 0.1% formic acid and acetonitrile with 0.1% formic acid respectively. The gradient elution starts with 1% B to 95% B over 14 min (22). The Orbitrap Fusion Mass Spectrometer fitted with heated electrospray ionization (HESI) was operated for positive mode (+ve) at 1,20,000 resolution in MS1 mode and 30000 resolution in data-dependent MS2 scan mode. The spray voltage used for these positive and negative modes is 4000 and 35000 volts respectively. Sheath gas and auxiliary gas were set to 42 and 11 respectively. The mass scan range was 50-1000 m/z, AGC (Automatic gain control) target at 200000 ions and maximum injection time was 80 ms for MS and AGC target was 20000 ions and maximum injection time 60 ms for MS-MS was used (22). Data processing was done using Thermo scientific Xcalibur software and the identification of metabolite was confirmed by accurate mass and MS/MS fragmentation match of the metabolites available from the literature and mzCloud database.

2.3 Predicting herb-drug interactions: an in-silico approach

The list of characterised phytochemicals was further used for predicting HDIs for safe and effective usage along with drugs used in the management of the COVID-19 and associated comorbidities. Thereby, the in silico pharmacokinetic studies were explored by using SwissADME tool (23).

2.4 CYP Inhibition Activity Assay

CYP3A4, 2C8, 2D6 inhibition activity assays were carried out by using pool of 50 human liver microsomes (HLM) having protein concentration of 10 mg/ml. Testosterone (70 μ M), paclitaxel (5 μ M), and dextromethorphan (5 μ M), are used as a probe substrate for CYP3A4,

2C8, and 2D6 respectively. The marker metabolites 6 β -hydroxy testosterone, 6-hydroxy paclitaxel, and dextrophan were used for the measuring the enzyme activity. For each isozyme, aliquot 99.5 μ L of incubation mixture (containing HLM and substrate) was spiked with 0.5 μ L of investigational plant drug/positive control/remdesivir working solution in a microcentrifuge tube and mixed by swirling and mild shaking. Thereafter, 10 μ L of 10 mM NADPH was added to initiate the reaction to all microcentrifuge tubes and incubated for 10 min at 37°C. The incubation reactions were stopped with addition of 400 μ L quenching solution (%100 methanol) (24). The samples were centrifuged at 10000 rpm for 5min. The supernatant was analysed by LC-MS/MS. The positive control inhibitor incubations were prepared similarly and analysed concurrently. Whereas the blank was prepared by spiking DMSO instead investigational plant drug. All the experiments were carried out in triplicates.

2.5 Preparation of test solution and positive control

The investigational plant drugs (*Aswagandha* and AYUSH-64) were prepared in 7 different concentrations ranging 2000, 1000, 100, 50, 20, 10, 1 μ g/ml. Stock solution of 500 mg/ml of investigational plant drugs was prepared with water and kept overnight in a mechanical shaker at a speed of 200 rpm at 37°C. The solutions were centrifuged at 10,000 rpm for 30 min and the supernatant collected was used for further analysis. The concentration ranges for positive controls were 5.00-0.0005 μ M, 1.00-0.01 μ M, 5.00-0.0005 μ M ketoconazole (CYP3A4) (24), rosiglitazone (CYP2C8) (25), and quinidine (CYP2D6) (26) respectively. The concentration of case control small molecule remdesivir were prepared in the range of 1000 – 1 ng/ml (27).

2.6 Measurement of marker metabolites by LC-MS/MS method

The marker metabolites (6- β -hydroxy testosterone, 6-hydroxy paclitaxel and dextrophan) formed as a result of metabolism by respective CYP3A4, CYP2D6, and CYP2C8 substrates (testosterone, paclitaxel, and dextromethorphan) were measured by using LC-MS/MS method.

The chromatography separations are carried out as mentioned above. The water with 0.1 % formic acid is used as mobile phase A and methanol and 0.1% formic acid as mobile phase B. The run time of 14 min with a flow rate of 0.300 ml/min were used. The method was followed as reported in the section 2.2

2.7. Data processing and targeted metabolite analysis

ThermoScientific Xcalibur system was used for data processing and data analysis. The standard metabolite retention time and MS-MS fragmentation was matched with sample. The respective metabolite concentration was monitored accordingly under different experimental conditions (CYP3A4, CYP2D6, and CYP2C8).

2.8 Determination of IC50 values

The above-mentioned concentrations of investigational herbal drugs (*Aswagandha*, AYUSH64) were selected on the basis of daily maximum human dose when diluted in 1 L of gastrointestinal fluid followed by distribution in 56 L of total body fluid (24). This assumption is based on Ayurvedic properties of these drugs and thereby considering the one compartment modelling distribution (28, 29). The percentage of (%) control activity and (%) inhibitory activity was calculated using the following formulae: % control activity = (peak area of metabolite formed in the presence of herbs/peak area of metabolite formed in control) × 100 and % inhibition activity = 100 – % control activity (30).

2.9 Prediction of probable clinical interactions

IC50 of CYP substrate (i.e., testosterone, paclitaxel, and dextromethorphan for CYP3A4, CYP2C8, and CYP2D6 respectively) activity in HLM was calculated graphically by nonlinear regression analysis of logarithmic inhibitor concentration (log conc.) versus % of inhibitory activity plot using GraphPad Prism 5. The data were expressed as mean ± standard deviation. Ratio of I/Ki was used to predict plausible clinical interactions and > 0.1 was considered for possible clinical interaction. [I] is mean maximum surrogate plasma concentration (C_{max}) at steady state after administration of the highest clinical dose of inhibitor in human and used from previously published literature. Inhibitory constant (Ki) was calculated using equation: $K_i = IC_{50}/1+[S]/[K_m]$. [S] and [Km] are substrate concentration and Michaelis constant respectively (24). Since the interaction between ketoconazole and testosterone, rosiglitazone and paclitaxel, quinidine and dextromethorphan are competitive. Therefore, in our experiment, [S] and [Km] values were same. [I] values for ketoconazole were used as 1 to 5 µg/mL (5 µg/mL was used in the experiment), likewise 100 µg/mL and 5 µg/mL for rosiglitazone and quinidine respectively. It was not possible to calculate I/Ki ratio for herbal extracts because of the absence of pharmacokinetics data (31). Instead, it was specified that the IC50 values of plant extracts <100 µg/mL or <100 µM/mL were considered to be a potent inhibitor of CYP450 enzymes which may results in undesirable HDIs (24, 32).

3. Results

3.1 Phytochemical characterization of *Aswagandha* and AYUSH-64 by using LC-MS/MS techniques

The plant phytoconstituents were characterised by accurate mass and MS-MS fragmentation pattern match. Briefly 11 and 24 plant phytoconstituents were identified from aqueous extract of *Aswagandha* and AYUSH-64 (Fig. 1, 2; S2 file; S3 file; S4.file.Tab.1, 2). The certificate of analyses of both the aqueous extracts showed presence of impurities (heavy metals) and microbial load are within the acceptable limit as per the pharmacopeial standards.

Table 1
IC50 of the studied herbal extracts *Withania somnifera* and AYUSH-64 on CYP3A4, 2C8, 2D6 isoenzyme system

Compound Name	CYP3A4	CYP2C8	CYP2D6
	IC50 (µg/ml)	IC50 (µg/ml)	IC50 (µg/ml)
<i>Withania somnifera</i>	528.1 ± 88.04	233.62±36.72	161.9±4.45
AYUSH-64	467.5 ± 78.61	63.44±14.9	216.25±39.5
Remdesivir + <i>Withania somnifera</i>	741.95±5.45	116.39±39.2	108.68±23.94
Remdesivir + AYUSH-64	201.92±13.18	85.21±18.3	111.05±5.07

Table 2
IC50 of the studied CYP substrate inhibitors and remdesivir on CYP3A4, 2C8, 2D6 isoenzyme system

CYP3A4				CYP2C8				CYP2D6			
Compound name	IC50	Ki	I/Ki	Compound name	IC50	Ki	I/Ki	Compound name	IC50	Ki	I/Ki
Ketoconazole (µM/ml)	0.348 ± 0.03	0.17	28.73	Rosiglitazone (µM/ml)	3.28 ± 0.21	1.6	60.9	Quinidine (µM/ml)	0.12 ± 0.001	0.06	83.3
Remdesivir (ng/ml)	291.13 ± 40.04	145.5	6.87	Remdesivir (ng/ml)	161.77 ± 8.38	80.8	12.3	Remdesivir (ng/ml)	327.25 ± 79.5	163.6	6.1

3.2 Prediction of herb-drug interaction studies: an in-silico approach

Previously published data on *Aswagandha* and its effect on CYPs (in silico & in vitro) have shown that, the aqueous extracts prepared as per procedure given in Ayurvedic text did not show inhibitory effects (12, 24, 32). Whereas, the *in-silico* approach was employed to predict pharmacokinetics of herbal extracts. The radar plots of phytoconstituents of AYUSH-64 showed that most of the compounds showed good oral bioavailability along with good drug likeliness and GI absorption (Fig. 3, S1.File, S4.File.Tab.3,4). In addition, few of the phytoconstituents of AYUSH-64 showed CYP3A4 inhibitory activity followed by inhibitory effect on rest of the CYP enzymes (CYP2D6, 2C8, 2C19, 2C9) to the lesser extent. This is helpful in predicting pharmacokinetic interactions and also to develop safe and effective dosage regimen. For instance, the CYP substrate (drugs) may show increased systemic concentration if their metabolising enzyme is getting inhibited.

3.3 Inhibitory effect on CYP isoenzyme systems

The effect of aqueous extracts of *Aswagandha*, AYUSH-64 and a case control small molecule remdesivir on CYP3A4, 2C8, and 2D6 were studied by using HLM. The inhibitory activity of CYP3A4, 2C8, and 2D6 was studied by using respective probe substrates namely testosterone, paclitaxel, and dextromethorphan respectively and the formation of corresponding metabolites 6-βhydroxy testosterone, 6-hydroxy paclitaxel, and dextrorphan was monitored at different concentrations by using LC-MS/MS method.

From the present study, the I/Ki ratio of ketoconazole (CYP3A4 inhibitor) was found to be 28.73. Whereas remdesivir was at 6.87, which showed moderate /weak inhibition of CYP3A4 compared to respective inhibitor (ketoconazole). The results are consistent with previous reports (Tab.2, Fig.4). On the other hand, *Aswagandha* and AYUSH-64 alone and in combination with remdesivir did not show any kind of CYP3A4 inhibition as their respective IC50 values are more than 100 µg/ml (Tab.1, Fig.4).

Similarly, the I/Ki ratio of rosiglitazone (CYP2C8 inhibitor) was 60.97. Whereas I/Ki ratio of remdesivir was 12.36 which might lead to a weak inhibition of CYP2C8 compared to respective the inhibitor (rosiglitazone) (Tab.2, Fig.5). Similarly, *Aswagandha* alone and in combination with remdesivir did not show any kind of inhibitory effect (IC50>100 µg/ml) towards CYP2C8 isoenzyme system. While, AYUSH-64 had moderate to weak inhibitory effect as the IC50 was above 100 µg/ml. Whereas, AYUSH-64 in combination with remdesivir (IC50= 85.21±18.3) followed the similar trend (Tab.1, Fig.5).

The I/Ki ratio of quinidine (CYP2D6 inhibitor) and remdesivir was 83.3, 6.1 respectively (Tab.2, Fig.6). The study demonstrates remdesivir to be a weak inhibitor of CYP2D6 as compared to respective inhibitor (quinidine). In addition, *Aswagandha* and AYUSH-64 alone and in combination with remdesivir did not exert any inhibitory effect on CYP2D6 isoenzyme as the IC50 value was >100 µg/ml (Tab.1, Fig.6).

The present study indicates that, both the AYUSH drugs seems to be safe to administer along with CYP3A4 and 2D6 substrates. *Aswagandha* also seems to be safe towards the respective CYP2C8 substrates. But AYUSH-64 has weak to moderate interactions. Hence the prescription should be under the observation of a physician / clinical pharmacist and care should be taken with the drugs that are taken concomitantly given with AYUSH-64.

4. Discussion

At present, although several drugs and therapies are in use to mitigate or to prevent from illness caused by SARS-CoV-2, still the urge towards developing the new medical strategies is needful (33, 34). The use of integrative medicine (IM) has become a trend either as a primary (to treat disease/illness) or secondary (to downgrade the prevalence of disease/illness) prevention (35). Moreover, the ideal therapeutic regime to treat COVID-19 should possess multi-targetability for immunomodulatory, adaptogenic, rejuvenating, anti-stress, anti-inflammatory, antiviral activity, etc (11, 12). It is also important for such IM pharmacotherapeutic regimen to be safe and efficacious to prevent or control the associated comorbidities. From the previous literature the identified phytochemicals from our study are already profound to elicit immune defence and anti-viral properties by which they might act as an adjuvant to treat COVID-19 (36). The usage of herbal extracts/formulations which are the mixture of large number of phytoconstituents renders to show multiple pharmacological activities as well untoward interactions when administered along with conventional medicines (herb-drug interactions)

(37). Such HDIs can be beneficial, harmful or even fatal. *Aswagandha* well-known *Rasayana*

(~rejuvenator) also referred as “Indian Ginseng” is well established for its immunomodulatory, adaptogenic, anticancer, anti-diabetic, anti-COVID-19 activities, etc (28).

In the present study we have characterized the aqueous extract of *Aswagandha* and found 11 important withanolide and withanoside glycosides were characterized based on their MS/MS spectra. These glycosides like withanoside VII, V, X are known to elicit immunomodulatory activity and antiviral activity (38). Similarly, AYUSH-64 a polyherbal formulation, AYUSH64 contains the aqueous extracts of *rasayana* botanicals namely, Saptaparna bark (*Alstonia scholaris* R. Br.), Katuki roots (*Picrorhiza kurroa* Royle ex. Benth), *Kiratatikta* whole-plant (*Swertia Chirata* Pexbex. Karst), and Kuberaksha seed (*Caesalpinia crista* L.) (39) was characterized with the help of MS/MS fragmentation pattern and 24 plant phytoconstituents were identified.

The *in-silico* pharmacokinetics of *Ashwagandha* and AYUSH-64 revealed that most of the key phytoconstituents of *Aswagandha* and AYUSH-64 showed good oral bioavailability, drug like properties and GI affinity. Whilst some of the bioactive compounds showed inhibitory affinity towards cytochrome P450 enzymes (CYP3A4, 2C8, 2C9, 2D6, 1A2). However, the low relative abundance of these molecules might be the reason for the IC50 value below 100 µg/mL. Thereby, the dose reduction may be required to have safe and beneficial HDIs. Whereas, opposite will be applicable for the prodrugs (39). Also, some of the docking studied also revealed the key phytoconstituents like withaferin A, viscosalactone B, withanolide A, vallesamine, neocaesalpin B, sweroside, picrine, β-Caesalpin were profound to show good docking core against COVID-19 (15, 39–41). Furthermore, to assure its safe usage in combination with other conventional drugs used in COVID-19 management and also to prescribe for the patients with other comorbidities, we performed the *in-vitro* herb drug interactions of aqueous extract of *Aswagandha* and AYUSH-64 by using human liver microsomes.

For instance, the usage of herbal medicines alone and also in combination of other conventional drugs were profoundly increased in the recent times (30). Hence, it is necessary to address their impact on cytochrome P450 enzymes (which contributes to the major hepatic metabolism of drugs) to understand their plausible metabolic interactions (42). In the present study, to simulate clinical context we incorporated to study the activity of herbs alone, as the selected herbs were already profound to be effective towards SARS-CoV-2. Besides, we also evaluated these extracts in combination with most commonly encountered drug remdesivir as a case example to understand their interaction with common CYP isozymes. For the same, the study design was focused on CYP enzymes that metabolise 60-80% of xenobiotic spectrum. CYP3A4 alone is responsible for the metabolism of more than 50% of all xenobiotics prescribed during various illnesses (43). Whereas CYP2C8 and CYP2D6 are responsible for 5% and 20% respectively (44, 45). Thereby, the prediction for CYP mediated inhibitory kind of HDIs for the drugs that are substrate of these CYPs can be done for the clinical translational purpose. The remdesivir was the most commonly prescribed for the COVID-19 pharmacotherapeutic management during the second wave of ongoing pandemic (46). It was therefore used as a case example for the *in-vitro* CYP based HDI studies. As individuals are mainly prone for the concomitant consumption of such herbal formulations with allopathic drugs, hence we also concomitantly subjected HLM to the remdesivir and herbal formulations at the same time.

Remdesivir is a substrate of CYP3A4, 2D6, 2C8. Results from our study indicated that $[I]/K_i$ ratio of remdesivir towards CYP3A4, 2D6, 2C8 was 38.07, 27.04, 16.62 respectively indicating that the specified case molecule showed poor inhibitory kind of potential which consistently supports the literature (47, 48). However, the aqueous extract of *Aswagandha* did not show any inhibitory potential, as the corresponding IC₅₀ values was >100 µg/ml towards all the three specified CYP isoenzymes (49). Interestingly this matches with the already published literature for Remdesivir, showing it as a weak inhibitor of CYP3A4 (50). Whereas for the *Aswagandha* our results match with the observations published by Patil *et. al.* showing aqueous extracts prepared as per Ayurvedic procedure does not show inhibitory effects on CYP3A4 (12, 24, 32). In addition, *Aswagandha* in combination with remdesivir exhibited IC₅₀ > 100 µg/ml which confirmed the weak inhibitory potential of remdesivir also in the presence aqueous extract of *Aswagandha*. This indicates that the combination of *Withania somnifera* and remdesivir, or any other drug which is the substrate of CYP3A4, 2D6, 2C8 might be safe to use for better pharmacotherapy.

Furthermore, we also identified the HDIs of polyherbal formulation (AYUSH-64) alone and in combination with the case control drug remdesivir. Results obtained signify that AYUSH-64 had no inhibitory interaction with CYP3A4 and CYP2D6 as IC₅₀ values were found be >100µg/ml. While, it showed a weak or moderate inhibitory interaction with CYP2C8 with an IC₅₀ of 63.44±14.9. It is therefore inferred that AYUSH-64 will be safe to administer with the substrates of CYP3A4 and 2D6, while a caution should be observed with drugs that are substrates of CYP2C8. Moreover, the action of AYUSH-64 in combination with remdesivir showed no inhibitory kind of interaction towards CYP3A4 and 2D6 with which IC₅₀ was >100µg/ml. As specified before both the AYUSH-64 and remdesivir showed weak or moderate interactions towards CYP2C8, the IC₅₀ of the combination showed 85.21±18.3. Indeed, this fall of IC₅₀ within the inhibitory limit confer that of these two together may competitively binds to substrate and showed moderate inhibition towards CYP2C8. The HDIs and pharmacokinetic parameters presented in the current study may provide insights to develop new and safe therapeutic regimen to mitigate SARS-CoV-2 and also provides safety profile to use concomitantly with other drugs. However, the study could not did focused on time dependant inhibition and induction type of Pharmacokinetic HDIs. Therefore, further, *in-vivo* and clinical HDIs studies should be performed in future for the translational understanding and better outcomes.

5. Conclusions

Generally, the combinatorial function of Ayurvedic extracts as an adjuvant with conventional drugs may produce beneficial effects. To make those favourable therapeutics outcomes to clinically practicable, it is necessary to have data regarding risks assessments (herb-drug interactions) for safe pharmacotherapy. The bioactive compounds of *Aswagandha* and AYUSH-64 which were already known to elicit immunomodulatory activity and antiviral properties might be suitable to prevent and treat COVID-19. *In-silico* study of the identified compounds showed CYP inhibitory affinity. Furthermore, the performed *in-vitro* CYP inhibition studies of plant extract along with case control drug (Remdesivir) revealed that *Aswagandha* may be safe to co-administer with the substrates of CYP3A4, 2C8, and 2D6. AYUSH-64 might not show any interactions towards CYP3A4, 2D6, while it might exhibit moderate interactions towards CYP2C8. Our study depicts the interactions of the herbs along with the case control drug that provides a practicable insight of their safe and concomitant administration in other comorbidities.

Declarations

6. Conflicts of Interest: None

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Figures

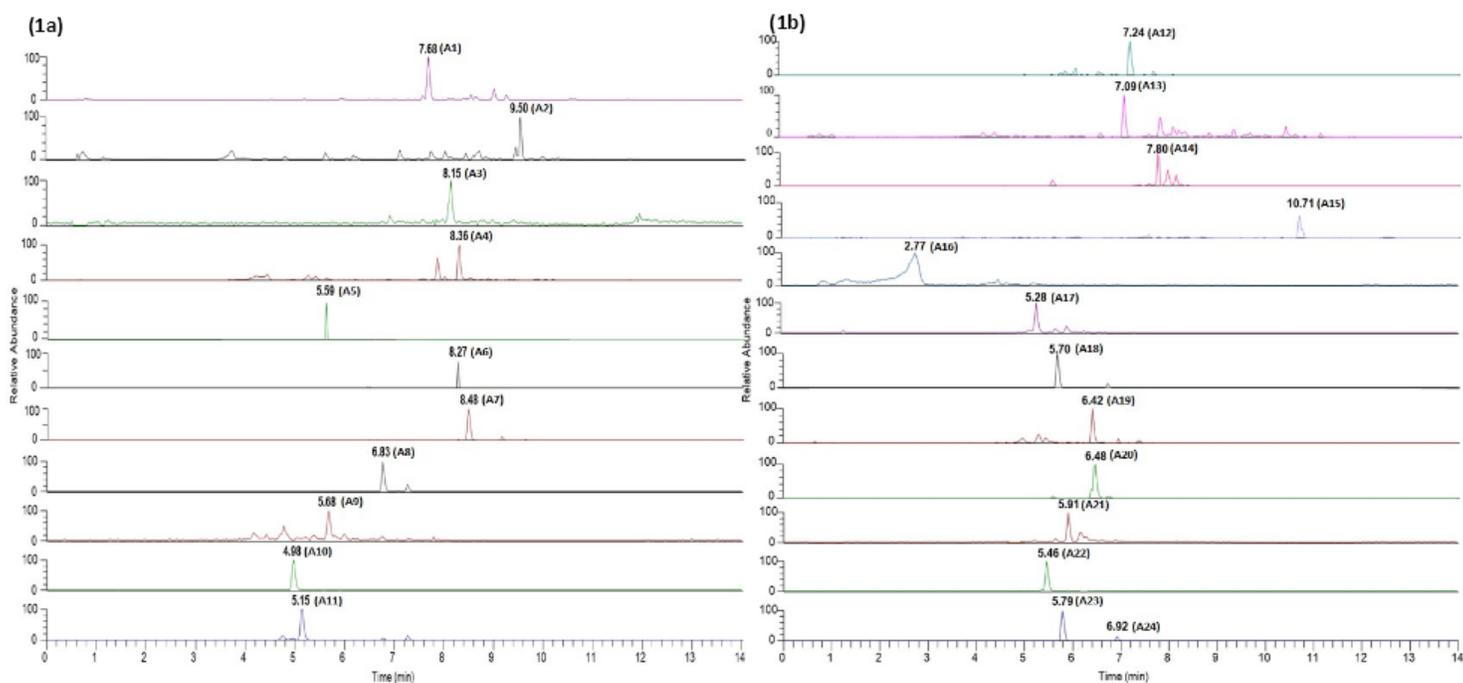


Figure 1

Extracted chromatograms of identified phytoconstituents of AYUSH-64. (1a) comprising compounds A (1-11); whereas, Betulin (A1), Neocaesalpin B (A2), Formononetin (A3), Burnamine (A4), Picrinine (A5), Vallesamine (A6), Oleanolic Acid (A7), Amarogentin (A8), Gentianine (A9), Mangiferin (A10), Sweroside (A11) and (1b) comprising A (12-24); Kaempferol (A12), 5-methoxystrictamine (A13), Neocaesalpin L (A14), Beta-Caesalpin (A15), Gallic acid (A16), Picoside B (A17), Verminoside (A18), Swertianolin (A19), Minecoside (A20), Apocynin (A21), Picoside-IV (A22), Kutkoside (A23), and Picoside-II (A24).

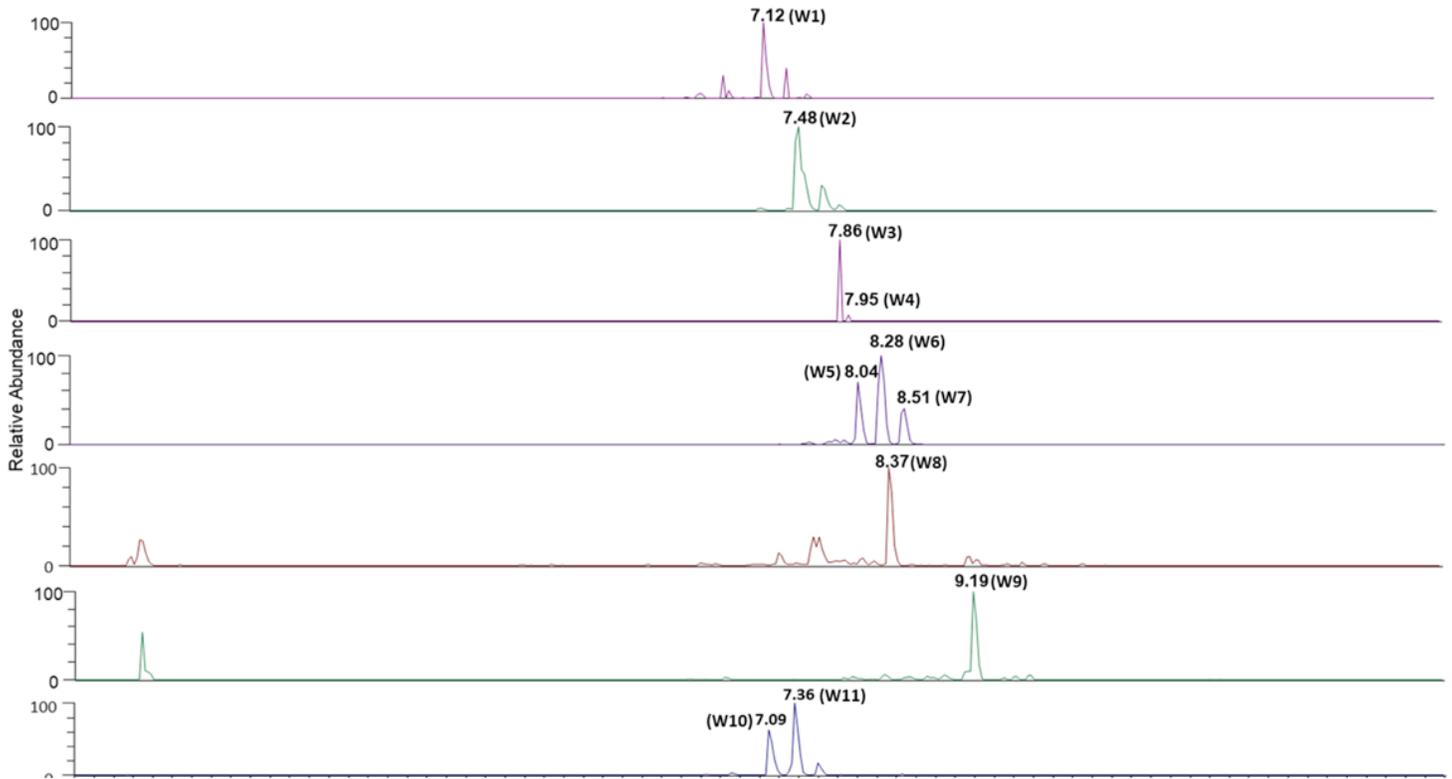


Figure 2

Extracted chromatograms of identified phytoconstituents of *Withania somnifera* comprising W (1-11); whereas, Withanoside VII (W1), Viscosalactone B (W2), Dihydrowithaferin A (W3), Withanoside V (W4), Withaferin A (W5), 12-deoxywithastramonolide (W6), Withanolide A (W7), 27-hydroxywithanone (W8), Withanolide B (W9), Withanoside X (W10), Coagulin Q (W11)

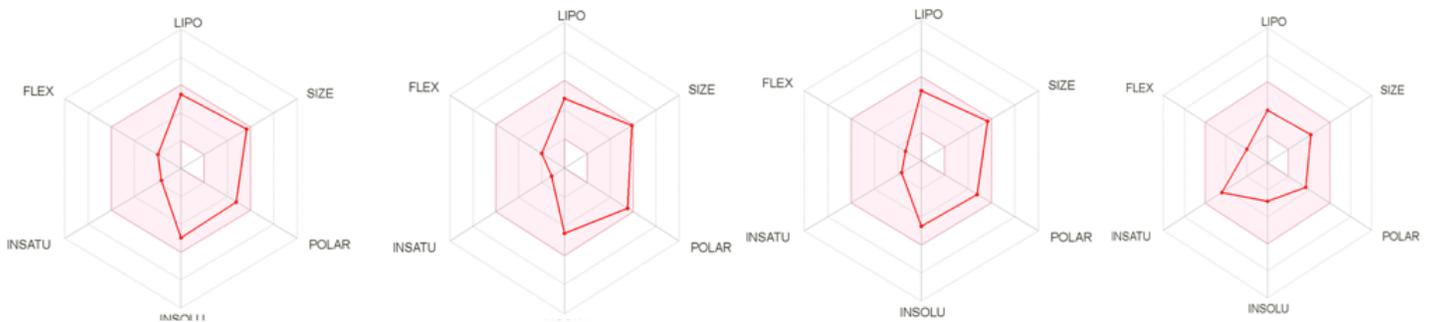


Figure 3

Illustrates the radar plots of key phytoconstituents identified from AYUSH-64 and *Withania somnifera*

Figure 4

Effect different concentrations of A) Ketoconazole (5.00-0.0005 $\mu\text{M}/\text{ml}$), B) Remdesivir (1000 – 1 ng/ml), C) Remdesivir + AYUSH-64 (2001- 1.001 $\mu\text{g}/\text{ml}$), D) AYUSH-64 (2000- 1 $\mu\text{g}/\text{ml}$), E) *Withania somnifera* (2000- 1 $\mu\text{g}/\text{ml}$), F) Remdesivir + *Withania somnifera* (2001- 1.001 $\mu\text{g}/\text{ml}$) on CYP3A4-mediated hydroxylation of testosterone. The graphs were plotted between log conc. Vs. percentage of inhibition.

Figure 5

Effect different concentrations of A) Rosiglitazone (100-0.01 $\mu\text{M}/\text{ml}$), B) Remdesivir (1000 – 1 ng/ml), C) Remdesivir + AYUSH-64 (2001- 1.001 $\mu\text{g}/\text{ml}$), D) AYUSH-64 (2000- 1 $\mu\text{g}/\text{ml}$), E) *Withania somnifera* (2000- 1 $\mu\text{g}/\text{ml}$), F) Remdesivir + *Withania somnifera* (2001- 1.001 $\mu\text{g}/\text{ml}$) on CYP2C8-mediated hydroxylation of paclitaxel. The graphs were plotted between log conc. Vs. percentage of inhibition.

Figure 6

Effect different concentrations of A) Quinidine (100-0.01 $\mu\text{M}/\text{ml}$), B) Remdesivir (1000 – 1 ng/ml), C) Remdesivir + AYUSH-64 (2001- 1.001 $\mu\text{g}/\text{ml}$), D) AYUSH-64 (2000- 1 $\mu\text{g}/\text{ml}$), E) *Withania somnifera* (2000- 1 $\mu\text{g}/\text{ml}$), F) Remdesivir + *Withania somnifera* (2001- 1.001 $\mu\text{g}/\text{ml}$) on CYP2D6-mediated hydroxylation of dextromethorphan. The graphs were plotted between log conc. Vs. percentage of inhibition.

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