Review

Andrographolide: A Herbal-Chemosynthetic Approach for Enhancing Immunity, Combating Viral Infections, and Its Implication on Human Health

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Abstract: Plants consistently synthesize and accumulate medically valuable secondary metabolites which can be isolated and clinically tested under in vitro conditions. An advancement with such important phytochemical production has been recognized and utilized as herbal drugs. Bioactive andrographolide (AGL; C_{20}H_{30}O_{5}) isolated from Andrographis paniculate (AP) (Kalmegh) is a diterpenoid lactones having multifunctional medicinal properties including anti-manic, anti-inflammatory, liver, and lung protective. AGL is known for its immunostimulant activity against a variety of microbial infections thereby, regulating classical and alternative macrophage activation, Ag-specific antibody production during immune disorder therapy. In vitro studies with AGL found it to be effective against multiple tumors, neuronal disorders, diabetes, pneumonia, fibrosis, and other diverse therapeutic misadventures. Generally, virus-based diseases like ZIKA, influenza A virus subtype (H1NI), Ebola (EBOV), Dengue (DENV), and coronavirus (COVID-19) epidemics have greatly increased scientific interest and demands to develop more effective and economical immunomodulating drugs with minimal side effects. Trials and in vitro pharmacological studies with AGL and medicinally beneficial herbs might contribute to benefit the human population without using chemical-based synthetic drugs. In this review, we have discussed the possible role of AGL as a promising herbal-chemo remedy during human diseases, viral infections and as an immunity booster.

Keywords: Andrographis paniculata (AP); andrographolide (AGL); anti-manic; anti-microbial; COVID-19 epidemic; immune booster; herbal-chemo remedy

1. Introduction

Andrographis paniculata (AP) a therapeutic herb, has been discovered and practiced as an effective herbal immuno-drug in traditional medicine systems to cure several health disorders worldwide [1]. Various scientific research has been conducted on the molecular and biochemical aspects of AP to improve the biosynthesis of its active ingredients i.e., andrographolide (AGL) [2]. The strong effects of AGL alike toxins, insecticides interfering with molting hormone pools-controlled insect pests thereby saving food crops with global economic benefit [3–5]. A decade of proliferating scientific research with AGL based on the active components established its multifarious pharmacological effect as an anti-bacterial, anti-viral, and anti-inflammation [6,7]. Till today the role of AP and AGL in human health has been explored and a long list of acute and chronic illnesses like infertility, diarrhea,
ischemia, pyrogenesis rheumatoid arthritis, obesity, upper respiratory tract infection, fever, hepatic and neural toxicity, cancer, etc., [8,9] signifies its role with relieving treatment history. Apart from many beneficial effects, AGL (in excess) may have harmful effects, but several scientific studies already focusing on such issues provided adequate data that it is still relatively safe. One such study with mice showed that a single oral AGL administration at a very high dose (2000 mg/kg) was not found to induce mortality despite altering the total body and organ weight, while no inflammatory responses and changes in hematological parameters were recorded [10]. It is also possible to administer AGL inside the cell using a carrier to further test its efficacy in cell culture and possibly to detect direct effects as mentioned in Sinha et al. [11] where the TaRKD-TALE protein was delivered in wheat microspores using a cell-penetrating peptide and expression regulation of microspore embryogenesis associated genes were monitored. This provides a reason for AGL to become a new scientific focus for researchers who intend to investigate its beneficial role in multiple diseases (mild, acute, and chronic) prevention, suppression, and therapy.

The therapeutic effect of AGL was tested by experimental and clinical research, which proved effective in overcoming microbial infections. Hua et al. [12] showed that AGL inhibits the growth of C. trachomatis bacteria which cause sexually transmitted disease and protects tissue from inflammation and damage. It significantly reduced the secretion of IL-6, IL-8/CXCL8 and interferon-γ-induced protein 10 being produced by the C. trachomatis infected host cells. Likewise, other researchers have also shown the inhibitory and suppressive effects of AGL on viruses such as human immunodeficiency virus (HIV) [13], herpes simplex virus type 1 (HSV-1) [13,14], hepatitis B (HBV) [15], hepatitis C (HCV) [16], and influenza virus [17]. Similarly, AGL was shown to be effective as an anti-viral agent against the Zika virus [18] and Chikungunya [19]. AGL has been used in large amounts to boost up immunity during viral outbreaks worldwide including the Indian dengue outbreak in 2006, where it was proved effective with a decrease in (+ve) cases and infection. The hypothesis was supported by the sequential in vitro studies through quantification of Dengue (DENV) inhibition being induced by AGL application [20]. Similarly, Ramalingam et al. [21] applied maximum nontoxic AGL dose and showed most of the antiviral inhibitory effects with DENV 1-4-infected Vero cells. Recently, Li et al. [18] evaluated the anti-viral activity of AGL against the Zika (ZIKV) and Dengue (DENV) viruses and confirmed their potential to be developed as an anti-ZIKV, anti-DENV herbal agents. Moreover, Kaushik et al. [22] used in vitro and in silico studies to characterize the isolated compounds from AGL with its active anti-dengue property against DENV-2.

Focusing on COVID-19, in silico study of Enmozhi et al. [23], Sharma et al. [24], proved that AGL functioned as a potential inhibitor of SARS-CoV-2 main protease. It also showed to have better inhibitory properties of proteases than other proposed inhibitors [25,26]. In various other examples, AGL and its derivatives have been used to successfully treat pediatric pneumonia and upper respiratory infections. While its combined application with other anti-inflammatory agents decreased the production of pro-inflammatory factors and cytokine secretion resulting in curative effects on upper respiratory tract infections [7]. AGL promoting the body’s immunity to achieve its curative effect against respiratory inflammation and Shigella dysenteriae is also well documented [27]. With such background in the present review, we aim to focus on the recent updates and the broad-spectrum effectiveness of AGL concerning human health specifically communicable diseases, microbial infections, immunity enhancement to fight against the viruses like COVID-19 and SARS-CoV-2. While the future prospective of AGL and its components as an effective chemo-herbal drug will also be discussed.

2. Functional Prospective of Andrographolide (AGL) on Human Health

In an immunocompromised patient, AGL intake plays a very important role by primarily initiating the immune response via modulating their complimentary system, granulocytes, and macrophages, which is useful in overcoming various diseases and infections (Figure 1).
2. Functional Prospective of Andrographolide (AGL) on Human Health... response was associated with the decreased tumor necrosis factor-α (TNF-α) which induced intercellular adhesion molecule-1 (ICAM-1) expression and adhesion of HL-60 cells into human umbilical vein endothelial cells (HUVEC). AGL can modulate the innate and adaptive immune responses by regulating macrophage phenotypic polarization and Ag-specific antibody production where MAPK and PI3K play an important role in macrophage activation and polarization. Such alteration through AGL can be achieved in different ways including the modulation of mRNA of inflammatory M1 macrophages, phenotypic alteration, functional alteration, and inducing the expression of alternative macrophages. This can cause a change in the expression of genes related to interleukins (IL4) and (IL13) which are involved in the...
macrophages metabolic pathways (Figure 2). The modulation of IL4 and IL13 not only affects macrophage but also ultimately affects the surrounding cells and tissues and thereby influencing the host defense and immunity. In the pathways, AGL can increase phosphorylation of p38 MAPK and inhibit the RIP2/Caspase-1/NF-κB [34]. Andrographolide application significantly reduced experimental autoimmune encephalomyelitis (EAE) symptoms in mice by impeding T-cell and antibody responses directed to myelin antigens (Figure 2). Results suggested that AGL can block T-cell activation in vitro and might be useful in the modulation of detrimental T-cell responses [35].

Figure 2. Schematic representation of the effects of andrographolide (AGL) and possible mechanism of its immunomodulatory regulation. Abbreviations: IL, interleukine; Th, T-helper cell type; MHS-1, major histocompatibility complex class II; CD, cluster of differentiation 80; CD86 cluster of differentiation; CD40, cluster of differentiation.

Exploring the beneficial effect of AGL on diabetes considering its worldwide propagation has always been of key priority. Diabetes-oriented research study showed that AGL scavenges the reactive oxygen species (ROS) and reduces the phenotypes of diabetic nephropathy (DN) in high glucose cultured MES-13 cells by intracellularly regulating the signal transduction pathway. Zhang et al. [36] showed that the application of AGL prevented the development of diabetes in autoimmune diabetic mice via strengthening its immune tolerance. Recently, a very interesting study by Su et al. [37] stated that AGL lowered the glucose effect via strengthening the function of the intestinal barrier, in-turns immune tolerance. With the advancement of therapeutic technologies, AGL nanoparticles (ANPs) are being in limelight today with their extraordinary adaptability, compatibility, bioavailability, and surprising loading capacity of the drugs. Undergoing constant modifications in their generation they were found to possess hepato-protective, anti-bacterial anti-malarial (anti-plasmodial), and anti-viral properties [39,40]. The increased anticancer efficiency of ANPs was found by Roy et al. [41] in mice model MCF-7 cells bearing experimentally induced Ehrlich ascites carcinoma. Sanati et al. [42] reported successful inhibition of neuroblastoma and cervical cancer cells by nano-encapsulation of the rich extracts of AGL. Further, AGL-loaded solid lipid nanoparticles inhibited head and neck cancer [43]. Andrographolide-
loaded nanocochleates were assessed for their physiochemical properties to be used as an oral delivery alternative for clinical trials in cancer therapy [44]. In addition, AGL nanoparticles were also tested and established to protect against cigarette smoking-induced chronic obstructive pulmonary disease [45]. It controlled aggressive asthma, where the pulmonary administration was reported to be more effective than oral administration [46]. Therefore, abovementioned functional prospects of AGL and its nanoparticles could be a boon to modern therapeutic practice with the possibility to participate in being a future herbal-chemo drug.

Andrographolide sulfonate (Andro-S) one of the derivatives of AGL is used in the treatments of inflammation-related diseases, however, Andro-S is effective in reducing acute lung injury (ALI). It was analyzed using an iTRAQ-based quantitative proteomics approach and by immunohistochemistry analysis [26]. The bioinformatic analysis of Gao et al. [26] revealed the inhibitory effects of Andro-S on lipopolysaccharide (LPS) induced ALI using potential targets as neutrophil elastase (ELANE), cathepsin G (CTSG), myeloperoxidase (MPO), and other three neutrophil-derived proteases. In this study, they assumed that the possible mechanism was by attenuating the expression of neutrophil-derived proteases, which may play a crucial role in the recovery of ALI. Such studies support a similar protective effect of Andro-S in reducing the ALI effect of Coronavirus (COVID-19) known to primarily target the respiratory tract.

Effectiveness of Andrographolide (AGL) with New Emerging Viruses and Their Frequent Mutant Counterparts (SARS-CoV-2 and COVID-19)

Recent pandemics with SARS-CoV-2 and COVID-19 accelerated studies targeting human immunity and defense against constantly emerging novel viruses. The severity of such pandemic accelerated grant funds from the World Health Organization (WHO), the European Union (EU), various governmental and research organizations supporting scientific research to develop effective drugs and vaccines capable of reducing worldwide infections and fatalities (October 2021, 4.96 M).

Development of new experimental methodologies is also in progress, where Chen et al. [47] constructed and validated SARS-CoV-2 drug target protein microarray and hypothesized it as a useful tool for pharmacological study. Khanit et al. [48] demonstrated AGL-induced anti-SARS-CoV-2 activity using Calu-3-based anti-SARS-CoV-2 plaque assay, where the effectiveness of AGL in SARS-CoV-2-infected Calu-3 cells of lung epithelial tissues was determined and AGL application significantly inhibited the production of infectious virions (0.034 µM; IC50—0.036 µg/mL). Researchers also advanced using molecular modeling and docking tool focusing on modulation of the immune system by inhibiting SARS-CoV-2 virus interaction with cellular receptors and thereover blocking NFκB1 and TNF pathway and restricting the COVID-19-induced cytokine storm responsible for the organ damage and mortality [49]. One such study showed that AGL exhibits binding affinity toward spike glycoprotein of SARS-CoV-2 and ACE2 receptor and therefore should be functionally explored as a therapeutic, prophylactic agent for restricting viral and host cell interactions [50]. Interestingly, Shi et al. [51] reported inhibition of main 2019-nCoV and SARS-CoV-2 proteases by covalent linkage with the application of AGL and its fluorescent derivatives.

With the background of 3D complex structure, SARS-CoV-2 S protein ectodomain binding to human ACE2 peptidase domain focusing on ACE2 inhibitors showed that AGL induced ACE2 receptor inhibition and its binding with the S protein of SARS-CoV-2 [52]. While Alazmi and Motwalli [53] proposed two natural origin S protein inhibitors (AGL and Pterostilbene) displaying better ACE-2 receptor and SARS-CoV-2 S protein binding potential thereon proving its beneficial property in attenuating the viral outbreaks. Further, Rajagopal et al. [54] through in silico approach explored the binding modes of AGL with the active site of SARS-CoV-2 main protease. Li et al. [55] using network bioinformatics analysis based on clinical knowledge prioritized AGL (among 30 candidates) as a potentially effective COVID-19 repurposable drug. Interestingly, Zhang et al. [56] in his clinical trial showed that injection with Xiyanping (XYP), a Chinese herbal compound (composed of
9-dehydro-17-hydro-andrographolide and sodium 9-dehydro-17-hydro-andrographolide-19-yl sulfate) improves and recovers COVID-19 patients having mild and moderate symptoms. Today, advancement with the laboratory and pharmacological studies focusing on AGL as a herbal bio-active compound has accelerated huge verified data with scientific outputs establishing its potential immune protective role in combating such viral outbreaks.

3. Modification of Andrographolide (AGL) and Its Constituents: Semi-Synthetic Antiviral Compounds

Bioengineering of active phyto-molecules and their components for human health benefits are the most emerging concept in the development of a herbal-chemo drug. Purposefully, the core structure of AGL was targeted and modified at various parts with emerging trends i.e., new structural features fitted to the receptors, the substitution of metabolically active compounds i.e., lipoic acid increasing the ability of the designed analog to reach the targets and induce selective biological activities thus, acting as pro-drugs. These modified analogs and derivatives were further in vitro tested for the treatment and prevention of a wide range of viral infections having mentioned below.

AGL and its constituents has been intensively investigated and established for antiviral activities against multiple viruses causing diseases including COVID viruses MERS-CoV, SARS-CoV, and flaviviruses [57–59]. 14-DDA, the most prominent analog of AGL was found to be active against several viral infections [60,61]. With modifications at C-3 by substituting OH to NOH and amides, a higher therapeutic index (TI) with moderate activity was recorded in comparison to the parent compound. 13,14-dihydroandrographolide alteration with g-lactone to N-methyl-g-lactam was highly effective against HIV, giving almost half activity of AGL with twice increase in the TI. Interestingly, the potency was found to be lost with the removal of N-methyl on the g-lactam. Whereas the best tested analog for anti-HIV activity was 3,19-di (acetoxy-benzyl)-isoandrographolide having TI more than 51 [62].

Many other constituents of AGL were found to be effective as an anti-viral and in prevention of the pre-infection like 12 and 14-acetylandrographolide, 14-deoxy-11, 14-acetyl-3,19-isopropylidenyl andrographolide, and 3,14,19-triacetyl andrographolide etc., [63,64]. Some of the AGL analogs and its derivatives with their activity level (CC50, EC50, SI/TI) on selective cell lines against the target viruses are shown in Table 1.

Table 1. AGL analogs and derivatives with their activity level (CC50, EC50, SI/TI) on selective cell lines, cytotoxicity assay against target viruses.

| AGL Analogues Derivatives | Virus | Targeted Cells | CC50 (µM) | EC50 (µM) | Cytotoxicity Assay | Selective Index (SI)/Therapeutic Index (TI) | Reference |
|---------------------------|-------|---------------|-----------|-----------|-------------------|---------------------------------------------|-----------|
| 14-aryloxy analogues ZAD-1 | ZIKV DENV | BHK-21 Vero | 136.3 ± 6 | 27.9 ± 1.7 | -                 | 9.8 (SI)                                    | [18]      |
|                           |       |               | 172.9 ± 22| 22.6 ± 1.8| MTT Assay        | 6.6 (SI)                                    |           |
|                           |       |               | 148.8 ± 40|           |                   |                                             |           |
| 14-aryloxy analogues ZAD-2 | ZIKV DENV | A549 | 217.7 ± 16 | 22.6 ± 1.8 | -                 | 179.2 ± 13                                  |           |
|                           |       | HEK293T/17    | 124.1 ± 18| 179.2 ± 17| MTT Assay        | 194.2 ± 17                                  |           |
|                           |       |               | 179.2 ± 13| 194.2 ± 17|                   | 196.8 ± 7                                   |           |
| 14-aryloxy analogues ZAD-3 | ZIKV DENV | HEK293T/17   | 175.0 ± 7 | 27.9 ± 1.7 | -                 | 160 ± 7                                     |           |
|                           |       |               | 186.1 ± 25| 22.6 ± 1.8| MTT Assay        | 190.1 ± 22                                  |           |
|                           |       |               | 201.4 ± 25|           |                   |                                             |           |
Table 1. Cont.

| AGL Analogues \ Derivatives | Virus | Targeted Cells | CC50 (µM) | EC50 (µM) | Cytotoxicity Assay | Selective Index (SI)/Therapeutic Index (TI) | Reference |
|-----------------------------|-------|----------------|-----------|-----------|-------------------|------------------------------------------|-----------|
| 3-O-Nicotinoyl-19-O-(n-decanoyl)-dehydroandrographolide | 3720 ± 725 | 142.2 ± 10.3 | MTT Assay | 26 (SI) | 165.1 (SI) | [14] |
| 3-O-Nicotinoyl-19-O-(1-naphthaleneacetyl)-dehydroandrographolide | >1706 | | | | | |
| 3-O-Nicotinoyl-19-O-(2-di-6-(furoic acid)-5,6-dihydroxyphenylacetyl)dehydroandrographolide | 2466 | 171.1 ± 13.5 | | | | |
| Human immunodeficiency virus | | | | | | |
| 3-O-Nicotinoyl-19-O-(3,4-dimethoxyphenylacetyl)dehydroandrographolide | 183 | 15.2 ± 4.09 | | | | |
| Human immunodeficiency virus | | 7.2 ± 1.5 | | | | |
| 14-deoxyandrographolide (IPAD) | 6.4 | | | | | |
| 3,19-isopropylideneandrographolide (DAD) | 80 | | | | | |
| 3,19-dipalmitoylandrographolide | 40 | | | | | |
| 3,14,19-triacetylamdrographolide | 4.2 | - | Anti-HSV-1 assay | | | |
| 14-dehydroandrographolide-12-sulfonic acid sodium salt (DASS) | 5.9 | | | | | |
| 14-a-lipoylandrographolide (AL-1) | 6.4 | | | | | |
| 19-O-(3',4',5'-Trimethoxy) cinnamoyl dehydroandrographolide | >1706 | 142.2 ± 10.3 | MTT Assay | 26 (SI) | 165.1 (SI) | [14] |
| 19-O-(2'-Thenoyl)-14-deoxy-14,15-didehydroandrographolide | 2466 | 171.1 ± 13.5 | | | | |
| 19-O-Nicotinoyl-19-O-deoxy-14,15-didehydroandrographolide | 183 | 15.2 ± 4.09 | | | | |
| 19-O-Cinnamoyl dehydroandrographolide | | 7.2 ± 1.5 | | | | |
| Human immunodeficiency virus | | | | | | |
| 3,19-(30-Nitrobenzylidene)-andrographolide | 745 | | | | | |
| 14-(20,60-Dichloro nicotinoyl) ester of andrographolide | 10354 | | | | | |
| 14-(1a)-Quinolinyl-5,7-dichloro-8-oxy)-19-acetoxyandrographolide | 88.7 ± 1.1 | 4.5 ± 0.2 | ZIKV titer assay | >16 | | |
| 14β-(8'-quinoloyl)-3,19-diol | 22.7 ± 1.1 | 1.3 ± 0.1 | | | | |
| 14β-Acetyl-3,19-diol | 20.8 ± 0.2 | 1.3 ± 0.5 | | | | |
| 14α-(8'-quino-linear-5,7-dichloro-8-oxy)-andrographolide | >100 | 13.3 ± 0.5 | | | | |
| 14α-(8'-quino-linear-5,7-dichloro-8-oxy)-andrographolide | 85.2 ± 1 | 7.8 ± 0.4 | | | | |
| Human immunodeficiency virus | | | | | | |
| 3,19-isopropylideneandrographolide (IPAD) | 39.71 | | Anti-HSV-1 assay | 2.20 (SI) | 2.34 | 2.32 | [18,68] |

Values with (±) represents the mean ± SD of three determinations. CC50 represents the minimum cytotoxic concentration that caused the reduction of viable cells by 50–80%. EC50 represents the minimum inhibitory concentration that reduced the cytopathic effect by 50%.

The corresponding oxime of 3-keto derivative, andrographolic acid amide derivatives, and 12-ester of 12-hydroxy-14-deoxy-13,14-dehydroandrographolide were tested against HIV with positive results [69]. Some modified analogs like 14-a-Lipoylandrographolide were effective against H9N2, H5N1, and HINI influenza as it inhibited viral adsorption into red blood cells by blocking cellular receptor bindings and interfering with viral haemagglutinin [17]. Hepatitis B virus (HBV) was successfully treated with the esterified derivative of 14-DDA at C-19 with pyridinecarboxylic acid or 2-thiophenic and furio acid by inhibiting DNA replication, HBsAg and HBeAg antigens [15]. During the anti-influenza virus study against H3N2, the benzyl amino derivative, 14-dihydroxy-17-(N-benzylamino)-7,13-ent-labdadien-15,16-olide showed the greatest potency being 1.5-fold more potent than the parent AGL [69]. When tested for anti-HBV activity with the structure–activity relationship the conjugated double bonds between C-11 and C-14 or C-12 and C-15, heterocyclic aromatic moieties and the free -OH was highly effective [15]. Genes and proteins
responsible for viral replications were found to be influenced by 3,19-isopropylidenyl andrographolide, which is probably due to the structural similarity with an anti-DNA replication compound 1,3-dioxolane [70]. Esterification of 11,12-didehydroandrographolide with succinic acid improved the activity against hepatitis C and H5N1 infections [71]. Today, AGL and its constituents are tremendously examined and getting established as semi-synthetic antiviral compounds with potential therapeutic effects.

4. Prospective of Herbal-Chemo Drug Development: A Potential Approach for Human Immunity Enhancement

The future of advanced pharmacological research should be confined to designing herbal supplemented synthetic chemo-therapeutic agents, which may prove not only immunosuppressive but also be combo-safe and can enhance molecular bioavailability and efficacy for better therapeutic outputs. Therefore, the identification of drug targets for AGL and its bioengineered components are of prime interest. Moreover, drug determination in biological samples is crucial to determine safer dose limits during effective drug development and discovery. Today the methods of separation, quantitative estimation of andrographolide (AGL) from a various herbal, complex mixture, and biological sources are established using spectrophotometry, chemiluminescence, electroanalytical and chromatography techniques [72] and being helpful in accurate detection and determination of its functional impact (Table 2).

| Source                                | Method/Instrument                                   | Solvent/Instrument Detection                                                                 | Limit of Detection (LOD)/Limit of Quantification (LOQ) | Reference |
|---------------------------------------|-----------------------------------------------------|-----------------------------------------------------------------------------------------------|-------------------------------------------------------|-----------|
| A. paniculate bulk powder             | UV/VIS Spectroscopy                                 | Methanol: water (50:50 v/v)                                                                | 1.2/4.23 µg                                            | [73]      |
| Herbs and herbal formulations         | FT/IR spectroscopy                                  | FTIR single reflectance horizontal ATR cell spectrometer (Perkin Elmer)                      | 1.0/3.34 µg/mL                                         | [75]      |
| Andrographolide in plant material     | FT/IR spectroscopy                                  | Perkin Elmer spectrometer with Kr-Optics and mercury cadmium telluride A detector            | 1.5/15 µg                                              | [76]      |
| Andrographolide Powder                | Flow-injection chemiluminescence                    | LC-20 A HPLC, fluorescence spectrophotometer, UV/VIS spectrophotometer                      | 0.0742 µg/mL                                           | [77]      |
| Andrographis tablets (Commercial)     | Cloud Point Extraction (CPE)                        | Triton X-114 (5%, w/v), 0.45 g NaCl, Agilent 1100 liquid chromatograph                      | 0.032 µg/mL                                             | [78]      |
| Human plasma (Andrographolide treated)| Cloud Point Extraction (CPE)                        | M.P.-methanol-acetonitrile-0.5% formic acid aqueous solution (40:17:43, v/v/v), SB C18 column (5 µM) | 0.75 µM                                               | [79]      |
| Human urine (Andrographolide treated), A. paniculata oil extract | Pulse Voltammetry Measurements                     | Autolab Pgtst 302 N Electrochemical system, Methrom Autolab B.V. (The Netherlands), pH meter, Boron-doped diamond electrode | 8.64–60.1 mg/L                                        | [81]      |
| Andrographolide (standards), dehydro-andrographolide commercial tablets. | Micromulsion electrokinetic chromatography (MEEKC) | SDS (15 mM) in 30 mM borate buffer pH 9.5. Waters (Milford, MA, USA) fixed wavelength with UV detector Quanta 4000E CE system | 0.30 and 1.0 µg/mL                                     | [80]      |
| Andrographolide (standards), dehydro-andrographolide commercial tablets. | Micellar Electrokinetic Chromatography (MEKC)        | SOD (15 mM) in 30 mM borate buffer pH 9.5. Waters (Milford, MA, USA) fixed wavelength with UV detector Quanta 4000E CE system | 8.64–60.1 mg/L                                       | [81]      |
Several pharmaceutical companies used herbal substances (e.g., curcuma, asafoetida, AGL) for drug product development, manufacturing, and executing the clinical trial development for various formulations. In this regard many Chinese formulas containing AP, AGL have been developed and patented as medicines with various formulations and drug dosages against the active viruses (www.cnki.net, accessed on 20 November 2021). Moreover, modulation of the immune response of these combo’s (herbal-synthetic agents) and their interaction with specific receptors and cellular components are being actively studied, monitored, and the data reveal the addition of their beneficial application [94]. Drugs like Remdesivir, Hydroxychloroquine, Captopril, Nafamostat in combination with AGL constituents are developed and patented as medicines with various formulations and drug dosages against HIV reproduction and in combination with multiple RT-inhibitor such as AZT (Zidovudine (Pro), Retrovir from Glaxo Wellcome) or DDI (Didanosine, Videx from Bristol-Meyers Squibb), antioxidants, vitamins, which are much affective in the modulation of CD4 (+) T lymphocyte behavior, in HIV management. Restomune, a natural product was approved by Health Protection Branch (HPB), Govt. of Canada (DIN 00774448) for the natural product “Restomune” in HIV management. Restomune, a natural product was approved by Health Protection Branch (HPB), Govt. of Canada (DIN 00774448) for the natural product “Restomune” in HIV management. Restomune, a natural product was shown by Basak et al. [99], showed the role of a new combination of drugs using the AGL-derived natural product “Restomune” in HIV management. Restomune, a natural product was approved by Health Protection Branch (HPB), Govt. of Canada (DIN 00774448) for the relief of colic/gastrointestinal gas disorder. The case report says that in some HIV cases Restomune as an alternative medicine boosted up the immune system by restricting the data reveal the addition of their beneficial application [94]. Drugs like Remdesivir, Hydroxychloroquine, Captopril, Nafamostat in combination with AGL constituents are developed and patented as medicines with various formulations and drug dosages against HIV reproduction and in combination with multiple RT-inhibitor such as AZT (Zidovudine (Pro), Retrovir from Glaxo Wellcome) or DDI (Didanosine, Videx from Bristol-Meyers Squibb), antioxidants, vitamins, which are much affective in the modulation of CD4 (+) T lymphocytes by viruses and finally preventing oxidative stress organ damage. It was also reported to promote CD4 (+) T-cell growth, which may compensate for the loss of CD4 (+) T cells at the time of fatal viral infection. In addition, the drug Restomune was also tested with a combination of other HIV-specific drugs and resulted in the rapid recovery of CD4 (+) T-cell counts. This combo-drug Restomune acts as an immune drug and significantly reduces

### Table 2. Cont.

| Source                                      | Method/Instrument | Solvent/Instrument Detection                          | Limit of Detection (LOD)/Limit of Quantification (LOQ) | Reference |
|---------------------------------------------|-------------------|------------------------------------------------------|------------------------------------------------------|-----------|
| A. paniculata (plant material)             | High-speed Counter-Current Chromatography (HSCCC)        | Multilayer coil counter-current chromatograph (Potomac, MD, USA), Water/methanol/ethyl acetate/n-hexane (2:5.2:5.4:1) 1.5 mL/min | -         | [82]      |
| Herbal extract and multi-herbal formulations | High-performance Thin-layer Chromatography (HPTLC)       | Silica gel 60 F254 Coated TLC Aluminum plates. Chloroform:Toxiroak:Meethanol (66:26:8, v/v/v) | 3.5 and 117 ng | [83]      |
| Polyherbal Livogat capsule,                |                   | Tolueno: Acetone:Formic acid (9:7.1, v/v/v)         | 62.91 and 209.7 ng per spot | [84]      |
| Bulk Drug and A. paniculata formulations determination. Andrographolide in Self-Nano Emulsifying Drug Delivery System (Sneeds) | High-performance liquid chromatography (HPLC)            | Isocratic methanol-Water (70:30) 0.8 mL/min, Xerra MS C 18 column (150 mm × 4.6 mm, 5 µm) | 1.95 and 3.13 µg/mL | [86]      |
| Andrographolide hypophyllanthin and phyllanthin (herbal liver protective formulations) |                   | Gradient-0.1% orthophosphoric acid (sol. A) and (1:1) acetonitrile: methanol (sol. B), Symmetry C8 column (250 mm × 4.6 mm, 5 µm) | 20 and 60 ng | [87]      |
| Touroak Premix (Polyherbal mycotoxin inhibitor) |                   | Isocratic acetonitrile:ortho-phosphoric acid (0.1%), 40:60 v/v 1.0 mL/min, C18 column Phenomenex Luna (250 mm × 4.6 mm, 5 µm) | 0.06 and 0.2 µg/mL | [88]      |
| Rat whole blood administered with Andrographolide containing liposomes and commercial tablets |                   | Isocratic methanol-Water (52:48 v/v) 0.8 mL/min, Chromasil ODS Column (25 mm × 4.6 mm, 5 µm) | 0.015 and 0.053 µg/mL | [89]      |
| Urine and feces of New Zealand rabbit’s (23187-INDUCED and treated with andrographolide) |                   | Isocratic methanol-water (55:45) 1 mL/min, 0.5 mL/min, C 18 column (250 mm in, 5-µm and 120 A pore size) | 1.87 and 5.45 µg/mL | [90]      |
| A. paniculata fresh leaves and stem         | Hyphenated technique | Gradient A:0.1% formic acid in water (B) 0.1% formic acid in acetonitrile 0.3 mL/min, Acquity BEH C18 (2.1 mm × 50 mm, 1.7 µm) | 0.18 and 0.75 ng/mL | [91]      |
| Pharmaco-kinetic analysis and distribution of andrographolide in rat tissues |                  | Gradient-2 mM ammonium acetate buffer (A), mixture of acetonitrile and solvent A (80:20, v/v) (B) 0.8 mL/min, C18 column (2 mm × 30 mm, 5 µm) | 3.91 ng/mL (LOQ) | [92]      |
| Human plasma determination of four major active diterpenoids from A. paniculata |                  | Gradient: water (A) acetonitrile(B) 0.5 mL/min, Kinetex column (4.6 mm × 150 mm, 2.6 µm) | 2.50 ng/mL (LOQ) | [93]      |
and enhances the immune system and SARS-CoV-2 [94,96]. Additionally, the examples of AP-based drugs and their active principles being successfully marketed are listed in Table 3. These combo multitargeted drugs benefit human health by relieving seasonal cold, fever, strengthening immunity, liver ailment, respiratory support, cardiovascular support, etc.

### Table 3. Bioactive extract of *Andrographis paniculata* (AP), its active principles from different commercial sources and their proposed functions on human health.

| Company/Manufacturer                      | Drug/Supplements               | Composition of the AGL Based Combo-Drug’s Active Principles | Proposed Functional Role |
|-------------------------------------------|--------------------------------|-------------------------------------------------------------|--------------------------|
| EU Nature                                 | Armor2 Andrographis Pure 800 MG| Andrographolides (200 mg), Gelatin                          | Strengthens immunity, seasonal protection, cold and flu |
| Bixa Botanicals                           | Andrographis                   | AP (Plant extract) 450 mg, 20% AGL, Gelatin                | Fever, liver ailments, blood sugar control, headache, anti-inflammation |
| Nine Life                                  | Andrographis                   | Andrographolides 900 mg, Gelatin, Rice Powder              | Supports healthy immune and liver function |
| Terry Naturally Vitamins                  | Andrographis and Ashwagandha   | AP (leaf extract) 200 mg, Ashwagandha (leaf and root extract) 150 mg, Hydroxypropyl methylcellulose, microcrystalline cellulose, magnesium stearate, silica, maltodextrin | Immune defence, antistress, energy and endurance, focus and clarity |
| Terry Naturally Vitamins                  | Andrographis EP80™ immune      | AP (Leaf extract) 60 mg, Melatonin 5 mg, Selenium 65 mg and Zn 15 mg Hydroxypropyl methylcellulose, microcrystalline cellulose, silica, cellulose powder, citric acid | Upper respiratory function support, cellular level support, restorative sleep |
| Terry Naturally Vitamins                  | Andrographis EP80™ Extra Strength| AP (Leaf extract) 40 mg, Hydroxypropyl methylcellulose, microcrystalline cellulose, magnesium stearate, silica | Immune function and upper respiratory tract health, joint health, daily energy and adaptability, intensive cellular health and DNA protection from oxidative stress, mental clarity and brain function |
| Terry Naturally Vitamins                  | Andrographis +Echinacea Vegan Capsules | AP (aerial extract) 200 mg, *Echinacea purpurea* root 200 mg | Immune system support |
| Solaray                                   | Andrographis aerial extract    | AP (aerial parts) 400 mg, Gelatin, microcrystalline cellulose, magnesium stearate, silica | Immune support, superior absorption, and bioavailability |
| Solaray                                   | Nano Andrographis Aqueous nano Andrographis 250 mg, rice flour | AP (aerial) 300 mg, AGL 4% Andrographolide methylcellulose, microcrystalline cellulose, magnesium stearate, silica | Immunity booster, respiratory tract benefit |
| Solaray                                   | Andrographis                  | AP (stem, leaf, flower) 100 mg, 10% AGL                     | Immune support, seasonal protection against cold and flu |
| Solaray                                   | Restenoril Andrographolide     | AP (stem) 500 mg, Gelatin, Methocel, Potassium sorbate | Supports a healthy immune response, promotes healthy cardiovascular function, provides antioxidant support |
| Solaray                                   | Andrographis Extract           | AP (leaf extract) 500 mg                                    | Liver detoxification, immunity booster, fights cold and flu |
5. Conclusions

In general, this review summarizes the therapeutic role of andrographolide (AGL) in boosting human immunity and treating diseases. The protective role of this multitarget bioactive herbal compound has long been established for treating microbial infections, fatal diseases like cancer, diabetes, acute respiratory tract infections, etc. AGL’s bioengineered analogs and derivatives with improved solubility, bioavailability, and therapeutic index (TI) is constantly synthesized and scrutinized providing directions for future therapeutic benefits. During the current global COVID-19 pandemic a lot of AGL-based productive research is on the go, yet a lot awaits to be explored at its target molecules and its constituents have a future for combo herbal-synthetic drug and may facilitate the pharmaceutical industry to design, validate, and produce AGL-based synthetic drugs targeting biological pathways for multiple therapeutic choices thereby increasing vitality and immunity.

Author Contributions: A.M. contributed to the conceptualization, designing, acquisition of data, and writing the original article with input from all the authors. H.A.S. contributed to visualization, writing, editing some sections and table preparations. R.K.S. performed the critical edition, figure preparation of the article. B.R.S. performed the editing and providing valuable outputs of the review article. All authors discussed the content and contributed to the revision and final version of the article. All authors have read and agreed to the published version of the manuscript.

Table 3. Cont.

| Company/Manufacturer | Drug/Supplements | Composition of the AGL Based Combo-Drug’s Active Principles | Proposed Functional Role |
|----------------------|------------------|-------------------------------------------------------------|--------------------------|
| Piping Rock          | AP Extract       | AGL (stem) 400 mg, Rice Powder, Gelatin Capsule, Vegetable Magnesium Stearate, Silica. | Immune support and liver function, dietary supplement |
| Oriental Botanicals  | ViraForce        | AGL 62.5 mg in combination with Olive leaf 1.25 g, Honeysuckle flower bud 1 g, Echinacea root 750 mg, vitamin C (250 mg), Zinc (8 mg). | Immunity against viral and bacterial infections, common cold, influenza (flu), tonsillitis and sinusitis, fever and headache, sore throat, and infections. |
| Fusion Health        | AntiViral        | AP leaf 4.5 g (ai 62.5 mg) combined with olive leaf (1250 mg), oleuropein (30 mg), Honeysuckle flower bud (1000 mg), Echinacea root 750 mg, Vitamin C (250 mg), Zinc glycinate (equiv. to 8 mg) | Antiviral and immune support during infections. Multiple disease and infections. |
| Andrographis         | Andrographis     | AP plant extract 500 mg combined with wood pulp, Colloidal silicon dioxide, Magnesium stearate, Dicalcium phosphate, Sodium benzoate | Liver support, multiple disease and infections. |
| Sears                | AntiViri         | AGL (95%), Taraxasterol (20%), Chlorogenic acid (25%), Lonerica Japonica (contains: standardized Chlorogenic acid 25%). | Boosts immunity, influenza, virus, COVID-19, SARS-CoV-1, SARS-CoV-2, anti-inflammatory properties, pulmonary protective |
| NHR Science          | Andrographis     | AP (leaf extract) 300 g; (Bioactive14-Neo-Andro Compound) Vegetarian capsules (Hydroxypropyl Methylcellulose), Non-GMO Rice Flour | Healthy inflammatory response, maintains bone mass and strength, immune response, supports nose, throat, and respiratory health |

5.1. Bioactive Herbs and Herbs in Combination

5.1.1. Adaptogenic Herbs

The use of adaptogenic herbs has gained increased attention in modern healthcare, mainly because of their ability to enhance resistance to stress and promote overall health. Adaptogenic herbs are known for their ability to regulate the body’s response to stress, support the immune system, and regulate hormonal functions. They are widely used in traditional medicine and have been integrated into contemporary healthcare systems around the world. Some of the most popular adaptogenic herbs include Ashwagandha, Rhodiola rosea, and Eleuthero (Siberian ginseng). These herbs are known for their ability to improve mental clarity, enhance physical endurance, and reduce stress and anxiety. Research has shown that these herbs can help reduce inflammation, boost the immune system, and improve overall well-being.

5.1.2. Antiviral Herbs

Several herbs have been found to possess antiviral properties, making them valuable additions to the repertoire of natural remedies. Some of the most widely recognized antiviral herbs include Andrographis paniculata, Echinacea, and Elderberry. These herbs are known for their ability to support the immune system, boost cellular defense mechanisms, and reduce the severity of viral infections. They are often combined with other herbs and supplements to create formulations that are more effective in preventing and treating viral illnesses.

For example, Andrographis paniculata, also known as Tinospora cordifolia, has a long history of use in traditional medicine for treating various infections and inflammatory conditions. Research has shown that this herb possesses potent antiviral and anti-inflammatory properties, making it a valuable addition to any natural remedy regimen. Similarly, Echinacea is a popular herb that is commonly used to support the immune system and reduce the duration and severity of cold and flu symptoms. Elderberry is another herb that has been widely used for its antiviral properties, and research has shown that it is effective in reducing the severity of cold and flu symptoms.

5.1.3. Immune-Boosting Formulations

Formulations that combine different herbs and nutrients have been shown to be effective in boosting the immune system. Some of the most popular formulations include PARACTIN® (https://cz.pipingrock.com/paractin-andrographis-paniculata-leaf-extract) and NHR Science (https://www.natures-source.com/products/paractin-andrographis-paniculata-leaf-extract), both of which contain Andrographis extract. These formulations are designed to support the immune system, boost cellular defense mechanisms, and reduce the severity of viral infections. They are often used in conjunction with other natural remedies to create a comprehensive approach to natural wellness and immune support.
**Funding:** The authors acknowledge funding from the Ministry of Education, Youth and Sports of the Czech Republic—CENAKVA project (LM20180099), Ministry of Education, Youth and Sports of the Czech Republic—the project Reproductive and Genetic Procedures for Preserving Fish Biodiversity and Aquaculture (CZ.02.1.01/0.0/0.0/16_025/0007370), Ministry of Education, Youth and Sports of the Czech Republic—the project Sustainable production of healthy fish in various aquaculture systems; PROFISH (CZ.02.1.01/0.0/0.0/16_019/000869), Ministry of Education, Youth and Sports of the Czech Republic (Project 821FR021) and Czech Academy of Sciences (RVO: 60077344).

**Conflicts of Interest:** The authors declare no conflict of interest.

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