

Chemical profiling of selected Ayurveda formulations recommended for Covid-19

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Abstract

Background: The novel coronavirus disease 2019 (COVID-19), caused by the Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is the global health concern since December 2019. It has become a big challenge for the researchers to find a solution for this newly evolved pandemic. In Ayurveda point of view, COVID-19 is a *Janapadodhwamsa vikara* (epidemic disease), a situation where the environment - air, water, land and seasons - is vitiated, causing a simultaneous manifestation of a disease among large populations. The aim of this study is to identify the active compounds of selected Ayurveda medicines recommended for covid-19.

Methods: Chemical profiling of selected Ayurveda medicines such as Indukantham Kwatham (IK), Vilvadi Gulika (VG) and Mukkamukkatuvadi Gulika (MMG) were carried out by High Performance Thin Layer Chromatographic (HPTLC) analysis.

Results: The selected preparations are traditionally recommended for the management of various kinds of fever including the infectious ones and to enhance the immunity. HPTLC analysis of the same showed presence of many active molecules like Umbelliferone, Scopoletin, Caffeic acid, Ferulic acid, Gallic acid, Piperin, Curcumin, Berberine and Palmatine.

Conclusion: The study provided valuable scientific data regarding the active ingredients of the selected medicines with proven therapeutic potentials like anti-viral, immunomodulatory and anti-inflammatory activities and it will be beneficial to acquire immunity against Coronavirus-2 attack.

Background

COVID-19 has emerged as the most dangerous global pandemic threat since its outbreak during December 2019 in Wuhan, China. As of August 12th, 2020, the World Health Organization (WHO) has reported more than 19 million confirmed cases and 7.3 lakhs deaths worldwide and it has spread to 216 countries, areas or territories [1]. Now, it is a big challenge for the researchers and health professionals to find out a solution for this deadly viral infection. COVID-19 is a viral infection that has been known to have the fastest frequency of replication in its positive strand resulting in the quick formation of new progeny viral cells inside the host cells. It has also been reported that SARS-CoV-2 has a high rate of mutagenesis and deviations in structure, which has formed a barrier for therapeutic procedures [2,3]. In Ayurveda point of view, COVID-19 is a *Janapadodhwamsa vikara* (epidemic disease), a situation where the environment - air, water, land and seasons - is vitiated, causing a simultaneous manifestation of a disease among large populations [4].

Medicinal plants have been used as a treatment and preventive strategy for several infectious diseases since ancient times. The advantage of using these herbs in viral respiratory infections is to build immune stimulating and inflammation modulating effects to prevent the severe life threatening conditions. Holistic approach of Ayurveda focuses on prevention of diseases through lifestyle modification, dietary management, prophylactic interventions for improving the immunity and managing the symptoms using

herbal preparations. Medicinal plants have been reported to have antiviral activity and many species such as *Aegle marmelos*, *Andrographis paniculata*, *Acacia nilotica*, *Ocimum tenuiflorum*, *Piper nigrum*, *Solanum nigrum*, *Terminalia chebula* etc. have been scientifically proved for their anti-viral properties [5-7]. Ayurveda medicines were recommended by Ministry of AYUSH, Government of India to enhance the immunity and to prevent the severe conditions of Cov-2 infection. Detailed guidelines have been published by the AYUSH ministry regarding the management of Covid-19. About 80% of COVID-19 cases are with mild symptoms requiring only primary medical care, 15% require urgent medical attention at secondary health care services and remaining 5% are life-threatening cases requiring an intensive care and hence require a transfer to tertiary health care units equipped with ICU. Ayurveda medicines are advised to patients with mild symptoms and those under surveillance which addresses the therapeutic province within an integrative model of care [8]. The present study was focused on identification of active ingredients of certain Ayurveda medicines such as Indukantham Kwatham (IK), Vilvadi Gulika (VG) and Mukkamukkatuvadi Gulika (MMG) in which the ingredient plants have been reported to possess immunomodulatory and anti-viral properties.

Indukantam kwatham is a Polyherbal tablet prepared out of specific parts of different medicinal plants such as *Holoptelea integrifolia*, *Cedrus deodara*, *Gmelina arborea*, *Aegle marmelos*, *Stereospermum colais*, *Oroxylum indicum*, *Premna corymbosa*, *Desmodium gangeticum*, *Pseudarthria viscida*, *Solanum anguivi*, *Solanum virginianum*, *Tribulus terrestris*, *Piper longum*, *Piper mullesua*, *Plumbago zeylanica* and *Zingiber officinale*. It is generally used for the treatment of intermittent fever and fatigue and to enhance the resistance power [9]. *Vilwadi Gulika* is prepared using different parts of the various medicinal plants such as *Aegle marmelos*, *Ocimum tenuiflorum*, *Pongamia pinnata*, *Veleriana jatamansi*, *Cedrus deodara*, *Terminalia chebula*, *Phyllanthus emblica*, *Terminalia bellirica*, *Zingiber officinale*, *Piper nigrum*, *Piper longum*, *Curcuma longa* and *Berberis aristata*. The ingredient plants of Mukkamukkatuvadi Gulika are *Terminalia chebula*, *Phyllanthus emblica*, *Terminalia bellirica*, *Zingiber officinale*, *Piper nigrum*, *Piper longum*, *Cuminum cyminum*, *Nigella sativa*, *Acorus calamus*, *Swertia chirata*, *Cinnamomum camphora*, *Myristica fragrans*, *Aloe vera*, *Syzygium aromaticum*, *Allium sativum*, *Piper cubeba*, *Saussurea costus*, *Cinnamomum verum*, *Ferula assa-foetida*, *Trchyspermum roxburghianum* and *Vitex negundo* [9].

Methods

Sample Preparation

The selected medicines were obtained from Product Development Department of Arya Vaidya Sala, Kottakkal, Kerala, India. Two gram each of IK (Batch No. 198339), VG (Batch No. 193083) and MMG (Batch No. 194967) were sonicated with chromatographic grade methanol in an ultra sound bath for 20 minutes. It was then filtered through membrane filter (0.45 µm) and kept under refrigerator until HPTLC analysis.

Instruments and general chromatographic conditions

HPTLC analysis was performed by CAMAG HPTLC system (Switzerland). Samples were applied using CAMAG ATS 4 auto sampler on aluminium backed pre-coated silica gel 60F₂₅₄ HPTLC plate (Merck India). Mobile phase was standardized as toluene, ethyl acetate and methanol in the ratio of 7:3:1. The chromatogram was developed in a saturated Twin Trough chromatographic chamber (Camag, Switzerland). The developed plate was visualized under UV at 254 nm and 366 nm and in visible light after derivetizing with Anisaldehyde sulphuric acid reagent followed by heating at 105°C for 5 minutes.

Results

Rapid chromatographic method has been developed for the chemical finger printing of selected medicines by modern high-performance thin layer chromatography. The optimized mobile phase provided good resolution under various documentation systems such as UV-254, 366 and visible light. Chromatogram and 3D illustrated display are presented in figure 1. HPTLC analysis showed presence of various compounds belonging to different groups of phytochemicals such as alkaloids, coumarins and phenolics. Structural identification was confirmed with matching R_f of standard compounds. IK showed presence of Piperine (R_f 0.71), Umbelliferone (R_f 0.61), Scopoletin (R_f 0.51), Caffeic acid (R_f 0.21), Ferulic acid (R_f 0.37) and Gallic acid (R_f 0.43). Compounds like Piperine (R_f 0.71), Umbelliferone (R_f 0.61), Scopoletin (R_f 0.51) and Gallic acid (R_f 0.43) were identified from MMG. Umbelliferone (R_f 0.57), Scopoletin (R_f 0.43), Caffeic acid (R_f 0.17), Ferulic acid (R_f 0.31), Gallic acid (R_f 0.43), Curcumin (R_f 0.73), Berberine (R_f 0.43) and Palmatine (R_f 0.33) have been identified from VG. Coumarins such as Umbelliferone and Scopoletin are detected in all the three selected medicines and these are the plant coumarins reported from many medicinal plants. Phenolic compounds like Caffeic acid, and Ferulic acid are found in both IK and VG. Gallic acid was identified from all the three selected medicines and has been reported from many ingredient plants. VG showed presence of alkaloids such as Curcumin, Berberine and Palmatine which might have extracted from its ingredient plants like *Piper nigrum*, *Piper longum*, *Curcuma longa* and *Berberis aristata*.

Discussion

Bioactive compounds from natural products are attractive candidates for drug development. Numerous medicinal plants have been reported to possess various therapeutic properties including anti-viral, anti-inflammatory and immunomodulatory activities. The chemical profiling of three selected medicines showed presence of various biologically active compounds belonging to different class of phytochemicals such as alkaloids, phenolics and coumarins. Coumarins such as Umbelliferone and Scopoletin were found to be common for all the selected samples. Naturally occurring coumarins have been reported to possess diverse biological and pharmacological properties such as anti-viral, anticoagulant, antibacterial, antifungal, antiprotozoal, insecticidal, fungicide, antimycobacterial, antimutagenic, anti-amnesic and anti-inflammatory activities [10,11]. There are numerous evidences for inhibitory role of coumarins against infection of various viruses such as HIV, Influenza, Enterovirus 71 (EV71) and coxsackievirus A16 (CVA16). The mechanisms involve either inhibition of proteins essential

for viral entry, replication and infection or regulation of cellular pathways such as Akt-Mtor (mammalian target of rapamycin), NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells), and anti-oxidative pathway including NrF-2 [12].

Alkaloids such as piperine, curcumin, berberine and palmatine were identified from the selected medicines. Piperine is detected from both IK and MMG and that might have come from the ingredient plant *Piper nigrum*. The immunomodulatory potential of piperine has been reported earlier [13]. Piperine was reported to inhibit proliferative response induced by lipopolysaccharide (LPS) and immunoglobulin α -IgM antibody and resulted in inhibition of IgM antibody secretion and reduced expression of cluster of differentiation CD86 [14]. Another study by Lee et al. 2018 [15], demonstrated that piperine in combination with gamma-aminobutyric acid (GABA) mediated p38 and JNK MAPK activation, which increased EPO and EPO-R expression, resulting in up-regulation of IL-10 and NF- κ B. Alkaloids like Curcumin, Berberine and Palmatine were identified from VG. The antiviral effect of Curcumin on Zika and Chikungunya viruses has been well established [16]. The literature showed that curcumin mediates its antiviral activity through various mechanisms. Curcumin has been reported to inhibit Japanese encephalitis virus by dysregulated ubiquitin-proteasome system and an accumulation of ubiquitinated proteins [17]. Curcumin was also reported to inhibit various virus replications like Rift Valley fever virus and Hepatitis C virus [18, 19]. Moreover, curcumin was shown to impact HCV replication through binding and fusion [20] and similar results were reported in the case of ZIKV and CHIKV. HIV-1 integrase activity of Curcumin was also reported previously [21]. Various pharmacological activities of Berberine such as anti-oxidant, anti-bacterial, anti-inflammatory, anti-viral, nephroprotective, cardio protective etc. have been reported earlier [22]. Antiviral Activity of Berberine against Human Cytomegalovirus has been reported previously [23]. The immunomodulatory effect of Berberine was validated in many previous literatures [24-26].

Currently, there are no available vaccines or specific medicines for the treatment of COVID-19. In light of the outbreak, various treatment modalities have been considered, including herbal medicine, which has been widely used during the past epidemic outbreaks, such as severe acute respiratory syndrome (SARS) and H1N1 influenza. The phytochemicals identified from IK, MMG and VG are active molecules with potential biological properties such as anti-viral, anti-inflammatory and immunomodulatory activities and it will be beneficial to enhance the immunity and to protect against the SARS Cov-2 attack.

Conclusion

COVID-19 pandemic is a global challenge for human health and researchers are urgently seeking medicine for it. Currently, the treatment options for Covid-19 are limited due to non-availability of vaccines or specific medicines. In this context, search for traditional herbal medicine is also a viable strategy for Covid-19 management. The present study on selected Ayurveda medicines provided valuable scientific data regarding the active ingredients of the same with proven therapeutic potentials like anti-viral, immunomodulatory and anti-inflammatory activities and it will be beneficial to acquire immunity against Coronavirus-2 attack.

Abbreviations

IK: Indukantham kwatham

VG: Vilvadi gulika

MMG: Mukkamukkatuvadi gulika

HPTLC: High performance thin layer chromatography

Declarations

Competing Interests:

The authors declare no competing interests

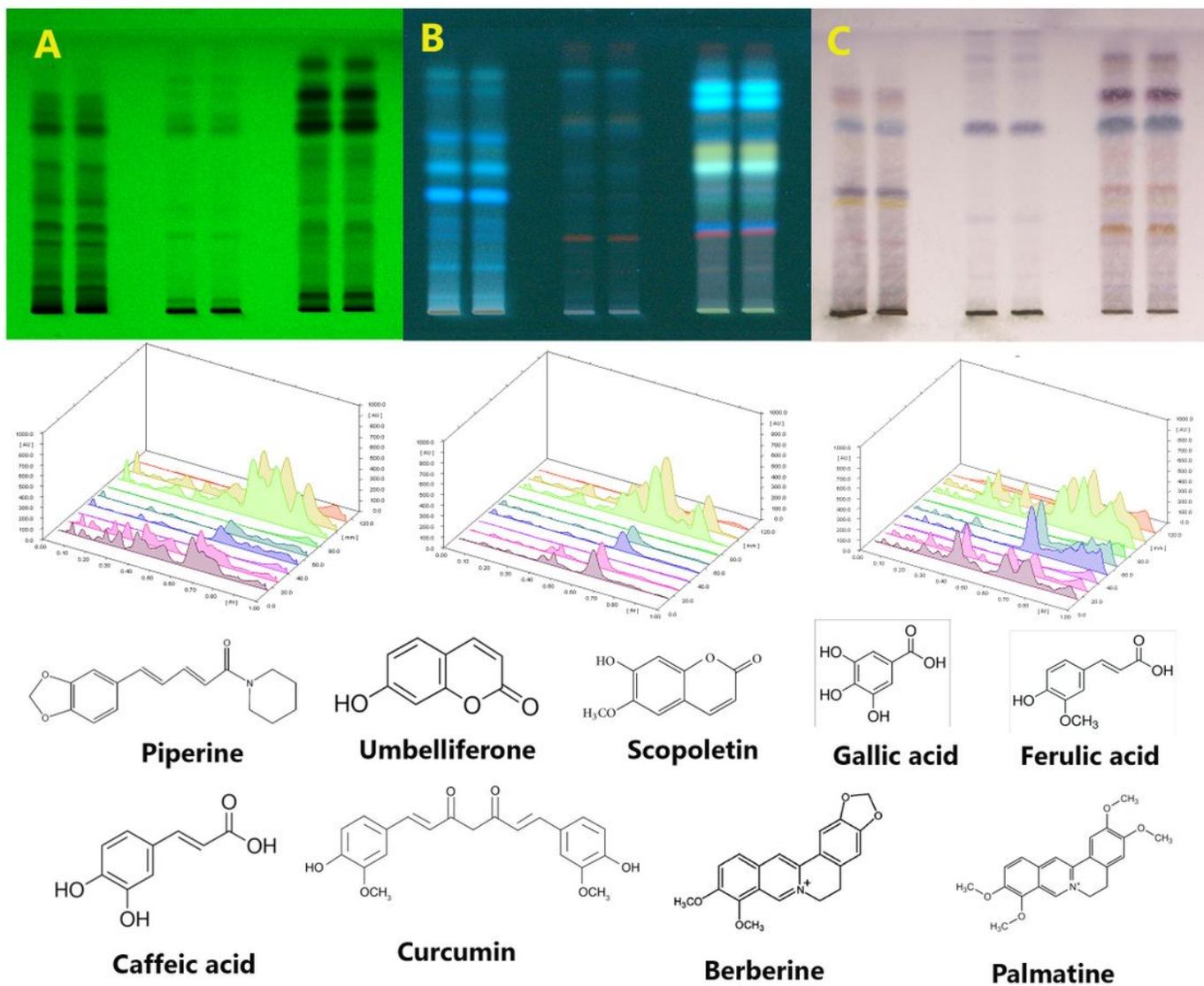
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Figures



Tracks: 1,2: IK; 3,4: MMG; 5,6: VG A: UV-254; B: UV-366; C: Visible light

Figure 1

HPTLC profiling of IK, MMG and VG documented at UV-254, 366 and visible light