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Analysis of Phytochemical Composition of Antivir-H and IMB Herbal Supplements Used for Management of Covid -19

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Abstract

The coronavirus disease (COVID -19) is a new zoonotic coronary system RNA virus that is highly infectious and has resulted in many deaths globally. Traditional medicines have been in use over decades in many parts of the World for management of different health disorders. The aim of this study was to carry out phytochemical analysis of two main herbal supplements registered by Kenya Pharmacy and Poisons Board and used for management of COVID-19 as herbal treatment named: Antiviral H and Immune Booster (IMB). An experimental study to evaluate the phytochemical profile of Antivir H and IMB herbal combinations of natural products was carried out in a laboratory to assess their phytochemical profiles. Mixed herbal powders for Antivir-H and IMB were screened to determine the presence of different classes of compounds. The powders were extracted in organic and inorganic solvents. The Thin Layer Chromatography (TLC) fingerprinting of the extracts were carried out to determine the phytochemical profiles. Results revealed that methanol-dichloromethane (DCM) of Antivir H had moderate presence of phenols, tannins and quinones while the aqueous extracts of the same show a high presence of flavonoids, phenols and saponins with moderate steroids. Methanol DCM extracts of IMB showed high levels of alkaloids with moderate phenols, tannins and quinones while aqueous extracts of IMB showed a high level of flavonoids, phenols and saponins with moderate tannins. In conclusion, most compounds were extractable using distilled water. However, the products are consumed wholesome orally and hence patients benefit from all the phytochemical compounds composed in the drug.

Keywords: Antivir-H, IMB, Phytochemical, Analysis, COVID - 19, Herbal Supplements.

Introduction

The coronavirus disease 2019 (COVID-19) is a pandemic which is caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has presented challenge in identifying efficacious drugs for its prevention and treatment (1). Given the speed at which scientific innovation and generation of clinical data by large number of people who have been infected and died of SARS-CoV-2, clinicians around the globe have worked to create evidence regarding effective treatments for the infection. There is rapid expansion of knowledge regarding SARS-CoV-2 virology that provides several potential drug targets to manage the disease, the most promising therapy being remdesivir. Remdesivir contains potent in-vitro activity against SARS-CoV-2. However, it is not approved in the US Food and Drug Administration (FDA) although it is currently under randomized trials. Oseltamivir is not efficacious and corticosteroids are currently not recommended. Current clinical evidence does not advocate for stopping angiotensin-converting enzyme inhibitors or angiotensin receptor blockers in patients with COVID-19.

COVID-19 is a new zoonotic coronary system RNA virus after MERS - CoV, and SARS- CoV and it is highly infectious, resulting in many deaths and causing anxiety around the globe. COVID-19 initial attachment is on the pharyngeal areas, progressing to rhinitis channels, sinus glands, tear glands and the oral-pharyngeal regions. The virus cultures on the pharyngeal glands for 3 - 4 days results into flu – like symptoms, sore throat as well as fever and

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coughing. From 5th to 14th day, the virus descends to the lungs, causing aggressive abrasions, degenerative effects and oxidative mechanisms which result to fibrosis, inflammation and fluid accumulation, eventually bringing shortness of breath. What kills patients infected with COVID-19 is Cytokine storm (2), of Acquired Respiratory Disease Syndrome (ARDS), a common immune-pathological outcome of SAS-CoV-1, SARS-CoV and MERS- CoV infections. The infection patterns progress to the lungs, liver, spleen and kidneys and in more severe cases, infection causes pneumonia, severe acute respiratory syndrome, kidney failure and eventual death.

COVID-19 pandemic poses a great global public health crisis since the pandemic influenza outbreak in 1918. The speed and volume of clinical trials launched to investigate potential therapies for COVID-19 propel need and capability of production of high-quality evidence of preventive and curative therapies. Search for control of COVID-19 at different laboratories include vaccines, concern being COVID-19 expresses and converts rapidly and this will prolong the realization of a working vaccine.

Other trials including antiretroviral drugs. DNA-RNA inhibitors and protease enzyme inhibitors have shown notable success, while others used chloroquine bisulphate or chloroquine diphosphate, a quinoline compound that aids in the intracellular transport for Zinc as an ionophore. The thinking was chloroquine triphosphates enabled zinc ionophore to accumulate and build in the infected cell nuclei, interfere with the biosynthesis of nucleic acids and curb viral expression and inhibit viral replication (3).

Traditional medicines have been in use over decades in many parts of the world to manage different health disorders. During the COVID-19 pandemic period, many trials for COVID-19 drugs and immune boosting products were undertaken in Africa and around the globe, with mixed results. Most of the trials initiatives in Africa have focused on herbal medicinal therapies (4). In Africa, some countries like Ghana, Mali, Nigeria and Rwanda have put in place a national policy, legal framework and budgetary allocation for Alternative Medicine Practice (5-7). For instance, Ghana has established centers for development of herbal medicine. In Madagascar, herbal medicines have been developed by Institute of Research in Herbal Medicine including diagnosis and prescribing done by doctors trained in herbal medicine (7, 8). A study was conducted in Nigeria by (9) on traditional medicine policy and regulation. The study revealed the existence of policy on regulation of traditional medicine (TM) but it has not been fully implemented, with argument that there is need to implement the guidelines to guarantee safety of TM use in Nigeria. All medicines in South Africa whether conventional and herbal are regulated by South Africa Health Products Regulatory Authority (SAHPRA).

Kenyan communities use traditional therapies for prevention of various ailments. Recently, combined use of traditional and conventional therapies in the management of emerging diseases has soared. The Kenyan government has instituted policy on the practice and use of Traditional Medicine, with the aim of enacting a framework for guiding areas for development and use of traditional and alternative therapies in Kenya (Traditional and Alternative Medicine Policy, 2018 Draft).

Antivir H: Antiretroviral (Antivir- H) is composed of Cetraria e., Licorice g., Warbugia u., Zanthoxylums c., Papain, Tylosema e., Vernonia a.,Oldefildea s., and Zinc supplement all composed and packaged in 500mg tablets. The Herbal Immune Booster (IMB) is composed of Licorice, Periwinkle, Sylibum m, Carrotene, Allium.s, Moringa.o, Carrisa e., Azadiracta, Chanca. p. and Nigella. s.

Materials and Methods

Materials / Implements

The herbal based Antiretroviral (Antivir- H) for antiviral mitigations and Immune booster IMB for boosting immunity and antioxidant effects combined are purposed to manage COVID -19 infections. The Antivir-H components include; Cetraria islandica lichen consisting of algae and fungus used for treating irritation of the mouth and throat, loss of appetite, common cold, dry cough, bronchitis, indigestion, fevers, lung disease, kidney and bladder complaints (10). Glycyrrhizin possesses anti-inflammatory and antioxidant activities and can stimulate endogenous production of interferons

(11). It has been shown to regulate pro-inflammatory mediators in CCl4-induced hepatotoxicity. Glycyrrhizin produces an inhibitory effect on HBeAg secretion that imparts anti-HBV activity. Anti-fibrotic activity of glycyrrhizin could be attributed to its inhibitory activity on NF-κB (ibid) showed that glycyrrhizic acid induces inhibitory effects on hepatocyte apoptosis and liver fibrosis, which was associated with down regulation of CTGF, HSC activation, MMP-2 and MMP-9, and collagen type I and III mRNA. These effects provide potential therapeutic strategies for antifibrosis (12). Warburgia u. has potential for the treatment of pneumonia, asthma, malaria, candidiasis, skin infections

Procedures (Phytochemical Analysis)

Sampling

For phytochemical analysis, mixed powdered materials were obtained from Pona Herbal Care Production laboratory in Kilifi in Kenya before they were pressed or packaged into tablets or capsules. They were packaged and availed at the Kenya Medical Research Institute (KEMRI) Centre for Traditional Medicine and Drug Research (CTMDR) for processing and analysis.

Aqueous solvents Extraction

About, 100g of the sample was weighed into a 1liter conical flask. One liter of sterile distilled water was added and mixed using a magnetic stirrer. The mixture was then put in a water bath set at 60°C so as to prepare concoction and left for 2-3 hours for full extraction to take place. Cotton wool and gauze roll was used to filter the concoction 2 times. About 200ml of filtrate was transferred to each round bottom flask and left overnight in a freezer (-8°C). Sample in round flask were freeze using acetone and dry ice to allow sample to solidify and attach on the walls of the flasks. The samples were finally put on to a freeze dryer (Edwards Modulyo Freeze dryer) for 5 days. The samples (dried) were then scrapped and yield weight recorded (Antivir-H; -12grams, 1MB; -l0grams).

Organic Solvent Extraction

About 100g of sample were weighed into a 1-liter conical flask. A ratio of 1:1 500ml of Dichloromethane and Methanol (DCM: MeoH) mixture was added to each of the samples shaken to mix and left to saturate for 72 hours thrice (maceration). The solution was then filtered with a WhattMann filter paper 1.2mm and the filtrate concentrated in vacuo using a Rotary evaporator (Butch Rota vapor R-300). The crude extracts were then allowed to dry for 3 days for all the solvents to get rid of traces of solvent before recording the yield.

Thin Layer Chromatography (TLC) Screening

For organic solvents, 2grams of the crude extracts were dissolved and spotted on aluminum-silica coated paper and was run in varying system ratios of hexane: ethyl acetate (9:1, 8:2, 7:3, and 5:5). Separation of compounds occurred and was observed under UV box, both long 36 nm, and short wavelength, 254nm. The separated spots were circled and the rf values were measured.

Qualitative phytochemical screening

Before the experiments were performed, all reagents were prepared freshly, ready for use. Five grams of extracts were dissolved in appropriate solvents of extraction. Tests for alkaloids, phenols, glycosides, saponins, tannins, terpeniods, steroids, quinones, and flavonoids were done and are displayed in Table 1 below.

Table 1: Phytochemical Screening

Phytochemical	Test	Procedure	Observation
Alkaloids	Dragendroff's Test	Few ml of filtrate + 1-2ml Dragendroff's reagents	A reddish-brown precipitate
Glycosides	Keller-Kikkan Test	1ml filtrate+1.5 ml glacial acetic acid+ 1 drop of 5% ferric chloride+conc H_2SO_4	A blue/green colored solution (in acetic acid layer)

Flavonoids	Ferric Chloride Test	Extract aqueous solution + few drops of 10% $FeCl_3$ solution.	A green precipitate
Phenol	Ferric Chloride test	Extract aqueous sol + few drops of 5% ferric chloride	Dark green/bluish black color
Tannins	10% NaOH test	0.4ml plant extract+4ml 10%NaOH +shake well	Formation of emulsion (hydrolysable tannins)
Steroids	Salkowski test	Filtrate +few drops of conc H_2SO_4 (Shake well and allow to stand)	Red colour in lower level
Quinones	Conc Hcl test	Plant extract +conc Hcl	Green color
Terpenoids'		Test tube containing chloroform add 0.5ml extract+3ml conc H_2SO_4	Reddish brown coloration of the interface

Results and Discussion

Results

The methanol-DCM extract of Antivir H showed moderate presence of phenols, tannins and quinones while the aqueous extracts showed a high presence of flavonoids, phenols and saponins with moderate steroids. The methanol DCM extract of IMB on methanol DCM showed a high presence of alkaloids with moderate presences of phenols, tannins and quinones while the aqueous extracts had a high presence of flavonoids, phenols and saponins with moderate tannins. The phytochemical analysis results are presented in Table 2 below.

Table 2: Results of phytochemical analysis of the organic and the inorganic extracts of ANTIVIR-H and IMB herbal products

	ANTIVIR-H MeOH/DCM	**IMB MeOH/DCM**	**ANTIVIR-H AQEOUS**	**IMB AQUEOUS**
Alkaloid	-	+++	-	+
Glycosides	+	++	-	-
Flavonoids	-	-	+++	+++
Phenols	++	++	+++	+++
Tannins	++	++	-	-
Steroids	+	+	++	++
Terpenoids	+	+	+	+
Quinones	++	++	-	-
Saponins	+	+	+++	+++
Key: + - Present but mild, ++ - Moderately present, +++ - Highly present				

Discussion

Both Antivir H and IMB herbal powders revealed the presence of Alkaloids, Glycosides, Flavonoids Phenols, Tannins, Steroids, Terpenoids, Quinones and Saponins at different amounts. The IMB herbal powder contained high amounts of (+++) Alkaloids (MeOH/DCM) and none in Antivir H when extracted using MeOH/DCM. In this study, the Alkaloids were found to be present in high quantities in both Antivir H and IMB. Alkaloids belong to the class of natural products that are known to have diverse pharmacological activity and great for the development of drugs for treatment of several pathologies including that of Covid-19. Silico studies demonstrated an affinity of the alkaloids for binding to the receptor-binding domain of the SARS-CoV-2 spike protein, putatively preventing it from binding to the host cell. Their study demonstrated that alkaloids have anti-coronavirus activity, hence a candidate for making bioactive agents against the SARS-CoV-2.

The study established the presence of more glycosides in IMB compared to Antivir H in the inorganic extracts. Flavonoids in both Antivir H and IMB were in equal amounts when extracted using aqueous solutions (13) in a study observed that glycosides compounds have antiviral and anti-inflammatory properties that have been in use in Chinese herbal therapy for centuries in several Asian counties for management of various ailments. Further, it has been established that geniposide, a main iridoid glycoside extract from the Gardenia jasminoides fruit, effectively blocks cells damage induced by pandemic [A/Jiangsu/1/2009 (H1N1)] influenza virus and alleviates virus-induced acute lung injuries (14). The glycoside found in the two products in this study is responsible for the bioactivities of the products against the virus causing COVID -19 disease. *Fructus Gardeniae* iridoid glycoside extracts (IGEs) exhibits antiviral effects against influenza A virus H1N1 and H3N2 subtypes *in vitro* and *in vivo* (15), this reveals that glycosides have some antiviral properties hence supporting the bioactivities of the products in the current study against the virus.

Phenols were in equal amounts in both Antivir H and IMB when extracted using aqueous solvents. The amounts of Phenols were variable in ANTIVIR-H when extracted using organic solvent MeOH/DCM, and in IMB using MeOH/DCM. Phenolics compounds are ubiquitous secondary metabolites found in plants and enhance antioxidant potential of a product. The phenols are aromatic compounds synthesized by phenylpropanoid pathway, and are plant-defenses mechanisms to pathogens, including bacteria, fungi, and viruses, and major abiotic stresses like drought, salinity, and Ultra Violet (UV) radiation. Phenolic compound exhibits antimicrobial and antioxidant properties, which help plants to evade pathogenic infections as well as to protect the major tissues from toxic effect of reactive oxygen species (16). Structural diversity of the phenolic compounds define their functional properties and the distribution for different plant species. Plants accumulate phenols at infection sites, which slow down the growth of microbial pathogens and restricts them at the infected site. Tannins were in equal amounts in both Antivir H and IMB when extracted using MeOH/DCM solvents. Tannins found in Antivir H and IMB are polyphenols enriched in wood, bark, roots, leaves, seeds and fruits of a variety of plants. Tannins have been shown to have possible anti-COVID-19 properties (13). Tannin in fruits and the existing knowledge on the activities of their compounds on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has potential of aiding scientists in the development of natural anti-viral chemicals for management of the current and possibly future pandemics (ibid). Therefore, anti-coronavirus properties of Antivir H and IMB could be due to the presence of tannins.

This study revealed that steroidal compounds in both Antivir H and IMB extracts were generally in low amounts. Steroids are naturally occurring compounds in plants and scientific evidence shows that corticosteroids help to relieve symptoms in different types of upper respiratory tract infections by acting to reduce inflammation on the lining of the nose thus improving the symptoms of common cold (17). Recent findings have demonstrated multiple physiological roles played by steroids during the course of different viral infections. However, practice does not support use of intranasal

corticosteroids in the symptomatic relief from the common cold (ibid).

This study revealed that terpenoids were the least present in both Antivir H and IMB organic and inorganic extracts. Triterpenoids are described as secondary plant metabolites that comprise six isoprene units. They are mainly found in higher plants and they have been studied for their structural diversity and presence of wide variety of bioactivities, including having antiviral, antioxidant, anticancer, and anti-inflammatory properties. Different triterpenoids have the potential to mimic antiviral agents (18). Antiviral activities of triterpenoids and the derivatives are examples of oleanane, ursane, lupane, dammarane, lanostane, and cycloartane triterpenoids. Triterpenoids have been shown to be important in defense mechanisms of plants against pathogenic infections and to exhibit excellent antiviral activities (ibid). Hence their presences in the products analyzed in this study are responsible for the antiviral bioactivities.

Quinones were highly present when both products were extracted using MeOH/DCM compared to water extraction in this study. Quinones are known to have some antibiotic properties against pneumonia causing pathogens. Quinone-based compounds make several classes of antibiotics, which have unwavering efficacy against infections caused by Gram-positive and Gram-negative bacteria (19). Quinone-based antibiotics are known to display different bioactive profiles depending on their structures and they exert specific biocidal and anti-biofilm properties (ibid).

The study demonstrated that, aqueous solutions of the two products each extracted equal amounts of saponins. Saponins occur naturally compounds in plants. Saponin from diets are shown to reduce cholesterol levels in the blood by binding to the cholesterol excreted in bile, therefore inhibiting entero hepatic cholesterol recycling, a mechanism through which saponins 'coat' cells to prevent attachment of virus and subsequent infection (20). Therefore, the two herbal supplements are possible traditional remedy for the treatment of COVID-19 because they have displayed a powerful antiviral effect against SARS-CoV. Further, it has been demonstrated from other studies that two viruses, which use different viral attachment proteins are blocked from binding to saponin-treated cells (ibid).

Conclusion

The study concludes that Antivir H and IMB contain phytochemicals able to exhibit antiviral activities that could be used to manage respiratory viral infections including SARS-CoV and COVID -19. Antivir H and IMB contain antibiotic properties useful for infections like pneumonia.

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Conflict of Interest

There is no conflict of interest in relation to this study from either parties involved.

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