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Review Article

A Review on Analytical Methods for Antiviral Phytoconstituents

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ABSTRACT

Viral infections are spreading to a great extent worldwide and affecting many people. The existing and emerging viral infections are a threat to human beings. Combating against these viral infections is a challenge with the available few antiviral drugs. Though new Antiviral drugs are being approved by the FDA every year, controlling emerging viral infections is a global concern. The currently available drugs and vaccines for the treatment of existing viral infections have limitations. From ancient times, herbs played a significant role in treating viral disorders by developing immunity towards viral infections. Some more herbs with their antipyretic and analgesic activity helped in treating fever and body aches resulted of viral infections. Antiviral phytoconstituents like alkaloids, glycosides, flavonoids, terpenoids, polyphenols, coumarins and saponins of plants have been isolated from plants and studied for their antiviral effects by researchers. Some of the phytochemicals are even developed as formulations and marketed to treat viral infections. Quality control of phytochemicals and herbal formulations is necessary for assuring therapeutic efficacy, quality and safety of these preparations. Hence, various standardization methods are developed for ensuring the quality of herbal products. The current review encompasses the RP- HPLC, LC-MS, GC-MS and HPTLC methods available for the Single and concurrent estimation of different antiviral phytoconstituents.

Key words: Analytical methods, Antiviral Activity, Phytoconstituents.

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INTRODUCTION

Most of the traditional systems of medicine are effective against many diseases, but standardization needs to be improved in certain stages of production. A number of new herbal products are introduced in the global market every year. 80% of the people in many parts of the world still rely on traditional herbal medicine and their products for their primary health care and living.[1] Many medicinal plant species have been recorded globally[2,3] In the present scenario more than 25% of the drugs are obtained from plant derived compounds.[4] Quality control methods need to be improved at every stage of production to ensure the quality, safety and efficacy. Hence, better standardization methods are needed to ensure the quality of products. Standardized herbal products of reliable quality and well defined phytoconstituents are required for clinical trials and to produce beneficial therapeutic efficacy.

The combination of high performance liquid chromatography and mass spectrometry (LC/MS) has a considerable impact on drug discovery and development of natural products. With the development of more sophisticated instrumentation and efficient column materials the HPLC and LC-MS techniques have now become more precise and reliable. Sometimes, the data generated from a single method is not enough to solve the problems of the confirmation of the structures of some molecules. The traditional method of extraction and isolation of these compounds using different chromatographic and spectroscopic methods such as TLC, column chromatography, flash chromatography, GC, HPLC, HPTLC, FTIR, NMR and MS have been progressively investigated to obtain and facilitate the identification of the bioactive compounds.[5]

The study design involves the development of new reverse phase HPLC and LC-MS methods for estimation of natural products, validation of the methods thus developed and testing their suitability for estimation of natural products. Chromatographic methods are very useful for the analysis of various classes of phytochemicals including alkaloids, coumarins, phenolic acids, flavonoids and isoflavonoids, terpenes and steroids. Phytocompounds play a crucial role in the field of drug discovery and development of antiviral agents with significant pharmacological effects.[6,7] Many anti-infective and anticancer drugs are derived from plant derived compounds[8] Herbal practitioners used herbal medicine since ancient times to cure several human aliments.[9] To treat innumerable inflictions and diseases.

Literature review on the analytical methods of some natural compounds by HPLC and LC-MS/MS, GC-MS and HPTLC methods are available for the estimation of natural products. The proposed methods can be less tedious and economical. The proposed methods can be used as alternative methods to those reported by the earlier workers and provide good choice for the routine determination of natural products. The current review encompasses the different method development and validation for the concurrent estimation of phytoconstituents. Since proper standardization methods are not available for herbal formulations this review gives an overview of Phytochemical compounds developed and validated by HPLC, LC-MS, HPTLC and GC-MS methods. Figure 1 represents the phytoconstituents and analytical methods.

Extraction and Isolation of Phytoconstituents

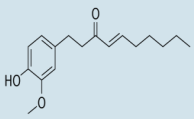
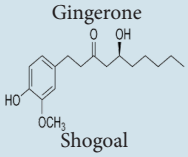
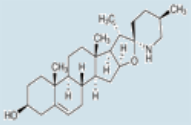
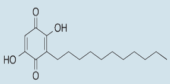
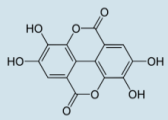
Many factors such as different solvents used for the extraction of bioactive molecules, choice of plant part and choice of solvents for extraction of bioactive compounds often play vital roles in extracting the biologically active phytoconstituents from plants effectively. To evaluate the antiviral activity of plants efficient approach for the extraction, isolation and characterization of bioactive molecules and virus replication inhibition assays in animals are definitely required before such phytoconstituents

could actually be employed to treat viral diseases.[10] Different methods for isolation, purification of bioactive compounds from the extracts of plant to carry out biological activity such as their antiviral, antibacterial, antifungal properties etc, are required to be established. Extraction of phytopharmaceuticals is a significant step in the analysis of plants to extract the desired Phytochemicals.[11,12]

Collecting information from local or ethnic group of people on usage of traditional and complementary alternative medicine using ethno medicinally important plants to extract bioactive compounds for curing various diseases or disorders are quite challenging. The pharmacological activity of herbal products depends on phytoconstituents present in them. Novel analytical methods can be developed and validated which can invariably outline the phytochemical compounds, including quantitative analyses of natural compounds and other major constituents, is a major challenge to scientists and researchers. Consistent therapeutic efficacy is not predictable without reliable quality of a phytochemical mixture. Chromatographic methods are relatively more useful techniques for qualitative and quantitative determination of drugs.[13,14] Different analytical methods for antiviral phytoconstituents are depicted in Table 1.

Medicinal plants play a valuable role in the field of research and development in exploring, extracting and establishing medicinal properties. Only very few phytoconstituents has been clinically evaluated for their therapeutic potential. Secondary metabolites which have been observed to combat viral infections include alkaloids, coumarins, flavonoids, lignins, polyphenols, proanthocyanidins, polysaccharides, saponins, quinones, terpenes, tannins, steroids and thiosulfonates are prominent bioactive phytochemicals. Medicinal Plants have natural tendency to treat various infections, diseases and disorders which have been nature's gift with rich source of phytoconstituents with wide therapeutic efficacy. Very few of the phytoconstituents have been standardized, purified and studied for their structural activity relationship and pharmacological effects. Most of the medicinal plant products have been marketed as herbal products without certified standardization, quality and efficacy. Many traditional medicinal plants and herbs have been reported to have strong antiviral activity against many viral infections. These

Table 1: Analytical methods for the Antiviral Phytoconstituents.

Biological Source	Phytoconstituents	Column used	Mobile phase	Analytical method
Zingiber officinalis  Gingerone  Shogoal	[6]-, [8]- and [10]-gingerol, [6]-, [8]- and [10]-shogaol, [6]-paradol and [1]-dehydrogingerdione	150 mm × 4.6 mm, 5 µm, Supelcosil™ LC_{18} column	The mobile phases consisted of solvent A (30 mM sodium phosphate buffer, pH 3.35) Solvent B (15 mM sodium phosphate buffer containing 58.5% acetonitrile and 12.5% tetrahydrofuran, pH 3.45).	HPLC[16]
Zingiber zerumbet L.	Sesquiterpenes (Zerumbone and α-humulene)	polydimethylsiloxane (PDMS, 7 µm), polyacrylate (PA, 85 µm)	70°C and 30 min	(HS-SPME) coupled with gas chromatography (GC-FID) HS-SPME-GC[17]
Zingiber officinale	6-gingerol, 6-shogaol, 8-gingerol, 8-shogaol, 10-gingerol, 10-shogaol, Zingerone and 6-isodehydrogingenone	Syncronis C_{18} column (100 × 2.1 mm, 1.7 µm)	The mobile phase consisted of acetonitrile and 0.1% formic acid in water	(UPLC-Q-Extractive–HRMS)[18]
Syzygium aromaticum	Eugenol 	cosmosil C_{18} column,	methanol: distilled water (60:40, v/v)	RPHPLC[19]
Solanum anguivi	Solasodine 	C_{18} column	methanol: KH2 PO4 buffer (pH 2.5) at the ratio of 75:25 %v/v	RP-HPLC[20]
Vidang *Embelia ribes Mallotus philippensis, Terminalia chebula*	Embelin, Rottlerin and Ellagic acid  	reversed phase C_{18} column eluted with gradient mobile phase of	The mobile phases consisted of acetonitrile and water	RP-HPLC[21]

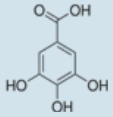
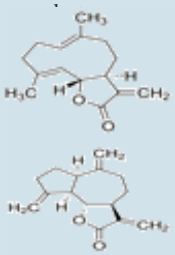
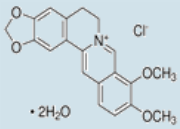
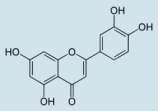
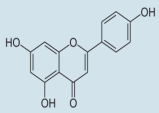
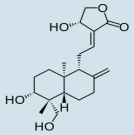
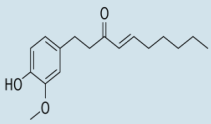
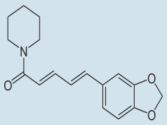
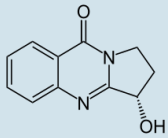
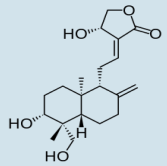
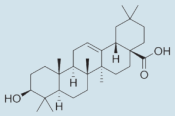
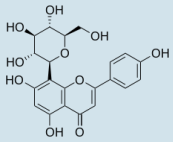
Triphala churna	Gallic acid, chebulagic acid and chebulinic acid as markers 	Phenomenex Luna C_{18} (2) column (250×4.6 mm id) 5 micron was used for separation.	Mobile phase A was potassium dihydrogen phosphate. Mobile Phase B was HPLC grade acetonitrile.	HPLC[22]
Saussurea costus	Costunolide and dehydrocostus 	Waters NOVAPAK HR C_{18} column (300 mm × 3.9 mm i.d., 6 µm)	Using isocratic elution with acetonitrile and water (60:40% v/v).	HPLC[23]
Tinospora cordifolia ***Tinospora sinensis***	Berberine 	C_{18} reverse phase column	acetonitrile: water (10:90 v/v)	TLC and HPLC[24]
Clerodendrum serratum 	Apigenin (API) and luteolin 	C_{18}G column (250 mm × 4.6 mm i.d, 5 µm)	methanol-acetonitrile-acetic acid-orthophosphoric acid-water (40:20:0.05:0.05:40)	RP-HPLC[25]
Andrographis paniculata ***Andrographis paniculata*** 	Diterpenes with andrographolide Andrographolide **1**, didehydroandrographolide **2**, neoandrographiside	C_{18} column monolithic Chromolith Performance RP C_{18} column	acetonitrile and acidified water water and methanol was used as mobile phase	HPLC[26] HPLC[27]
Andrographis paniculata	Diterpenoids andrographolide, 14-deoxy-11,12-didehydroandrographolide, 14-deoxyandrographolide and neoandrographolide	C_{18} column	methanol-water (55:45, v/v)	HPLC[28]

Table 2: HPTLC methods for the Antiviral Phytoconstituents.

Biological Source	Phytoconstituents	Retention Factor	Mobile phase	Analytical method
Zingiber officinale	8-gingerol 	retention factor (R_f) value of (0.39±0.04)	n-hexane: ethyl acetate 60: 40 (v/v) UV densitometric scanning at 569	HPTLC[29]
Zingiber officinale	Gingerone 6-SHO and 6-GIN	R_f = 0.36 ± 0.01 for 6-SHO and R_f = 0.53 ± 0.01 for 6-GIN	ethanol:water (6.5:3.5 *v/v*)	HPTLC[30]
***Piper nigrum* L.,**	Piperine 	337 nm	toluene-ethyl acetate-diethyl ether 6:3:1 as mobile phase	HPTLC[31]
***Piper nigrum* L.,**	Piperine and piperlongumine	R_f 0.51 and 0.74, 342 and 325 nm	toluene: ethyl acetate (6:4, v/v)	HPTLC[32]

Adhatoda vasica	Vasicinone 		toluene:butanol:butyl acetate (9:0.5:0.5; *v/v/v*)	HPTLC[33]
Andrographis paniculata* and *Eclipta alba	Andrographolide and wedelolactone 	254 nm	toluene:acetone:formic acid (9:6:1)	HPTLC[34]
Cissampelos pareria	Oleanolic acid 	530 nm	toluene: ethyl acetate: formic acid (7:3:0.3, %v/v)	HPTLC[35]
Passiflora foetida	Vitexin 		ethyl acetate:methanol:water:formic acid 30:4:2:1(%, *v/v/v/v*)	HPTLC[36]

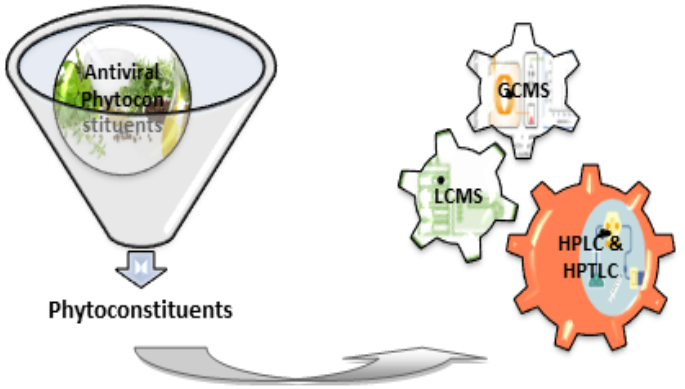


Figure 1: Phytoconstituents and Analytical Methods.[56-58]

phytoconstituents must be subjected to preclinical studies and human clinical trials to determine their effectiveness in whole-organism systems and toxicity studies.[15] HPTLC methods for the Antiviral Phytoconstituents are given in Table 2.

Antiviral Phytoconstituents of Pharmaceutical Importance

Antiviral activity of Flavonoids

Flavonoids are natural products belonging to plant secondary metabolites they are compounds having a polyphenolic structure present in fruits, vegetables and medicinal plants. They have antioxidant free radical scavenging activity associated with various diseases such as viral infections, cardiovascular diseases, anti-cancer, neurological disorders, skin diseases and various other acute and chronic diseases. Flavonoids are class of bioactive compounds having potent anti-viral, antioxidant and anti-carcinogenic potential. Flavonoids can be extracted and isolated from fruits, colored pigments, vegetables and medicinal plants. They are potent active constituents present in fruits and vegetables flavonoids are a group of phenolic compounds which are categorized into flavonols, flavones, catechins, flavonones, anthocyanidins, isoflavonoids, coumarins and chalcones etc. Recent studies are proven to be done to enhance the efficacy and bioavailability of flavonoids. Flavonoids are proven to be most effective against many diseases. These flavonoids are compounds which have immunomodulatory activity which can boost our immune system and prevent the body against many diseases like viral, cardiovascular, cancer, neuroprotective and so on.

Antiviral activity of Alkaloids

Alkaloids are secondary metabolites of plants origin consisting of nitrogen atom in the heterocyclic ring. Alkaloids are generally present in plants and comprise of various biological activities like antiviral, anti-inflammatory, anticancer, antibacterial, etc. Alkaloids have been observed to exhibit better therapeutic activity against various viral infections. The antiviral activity against virus by the alkaloids can be due to the immunomodulatory activity of phytoconstituents.[37]

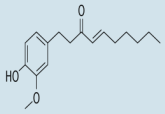
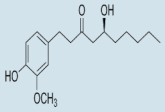
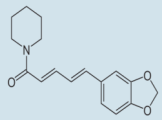
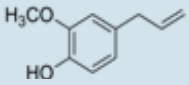
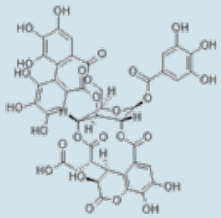
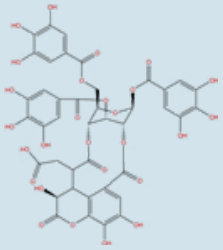
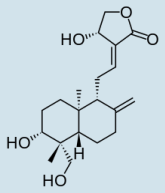
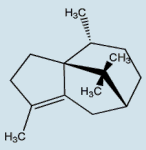
Antiviral activity of Terpenoids

Terpenes consist of five carbon (C_5H_8) compounds, isoprene units with hydrocarbons, obtained from isoprenoid biosynthesis and belong to the largest class of secondary metabolites. Terpenoids are class of compounds present in natural products with various pharmacological activities. Terpenoids are obtained from isoprenoid compounds present in plants and have a significant role in treating viral infections, as phytopharmaceuticals with better therapeutic activity.[37] Table 3 specifies Antiviral Phytoconstituents of Pharmaceutical Importance.

CONCLUSION AND FUTURE PERSPECTIVES

Antiviral phytoconstituents play a critical role in curing and treating viral infections. Available antiviral medicines from ancient system of medicine such as Ayurveda, Siddha, Unani and Homeopathy are being used as alternative medicines against viral infections.

Table 3: Antiviral Phytoconstituents of Pharmaceutical Importance.

Plant Name	Structure	Phytoconstituents	Cells	Activity
Zingiber officinale		Gingerol and Shoagol	HEp-2 and A549 cell lines	Human respiratory syncytial virus (HRSV).[38]
		Gingerol and Shoagol	Vero cell-line MTT assay	Chikungunya[39]
		[6]-gingerol and [6]-paradol	Hepatocellular carcinoma HepG2 cell line infected with HCV	Hepatitis C virus (HCV).[40]
Piper longum		Piperine	Hep G 2.2.15 cell line *in vitro*	Anti-HBV activity *in vitro*[41]
		Piperine	Coxsackie virus type B3 (CVB3)	Human rhinovirus type 2 (HRV2) and influenza virus type A (HK68).[42]
Syzygium aromaticum		Silver nanoparticles		Newcastle Viral Disease (NDV).[43]
		Eugenol	Host cells of FCV Crandell-Reese feline kidney (CRFK) cells	human norovirus[44]
Anacyclus pyrethrum				Immunostimulant activity[45]
Solanum nigrum		Solasodine	HCV NS3 protease	anti-HCV activity[46]
Terminalia chebula		Chebulagic acid and chebulinic acid	Vero cells by MTT assay	Herpes simplex virus-2 (HSV-2)[47]
		Chebulagic acid punicalagin		Herpes simplex virus type 1 (HSV-1) human cytomegalovirus (HCMV), hepatitis C virus (HCV), dengue virus (DENV), measles virus (MV) and respiratory syncytial virus (RSV)[48]
		Chebulagic acid (CHLA) and chebulinic acid		Influenza A virus (IAV)[49]
Saussurea lappa				Hepatitis B virus (HBV)[50]
Andrographis paniculata		Andrographolide	A549 cells	Antiviral and immunostimulant[51]
		Andrographolide		Chikungunya virus[52]
		Andrographolide	Vero cells *in vitro*	anti-dengu viral activity[53]
***Cissampelos pariera* Linn**			AG129 mouse model	pan-DENV inhibitory activity[54]
Cyperus rotundus		Cyperene	HepG2 cells	Hepatitis B virus (HBV)[55]

Several standardized protocols are available on authentication of herbs, isolation, characterization and estimation of active antiviral phytochemicals. Generally, in herbs active phytoconstituents exist along with other complex mixtures of compounds. Highly sensitive and selective spectroscopic and chromatographic methods are widely in use for identification and characterization of antiviral phytochemicals to overcome the interference of other constituents present in herbal formulations. Analytical methods such as RP-HPLC, HPTLC and hyphenated analytical methods like HPLC-MS/NMR, LC-MS and LC-NMR have been established as significant selective tools in the estimation of antiviral phytochemicals. Standardization and quality control of antiviral phytochemicals could be improved by using these sensitive analytical methods.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

TLC: Thin layer chromatography; **NMR:** Nuclear Magnetic Resonance; **LC-MS/MS:** Liquid chromatography mass spectroscopy; **HPLC:** High performance liquid chromatography; **HPTLC:** High performance thin layer chromatography; **UFLC:** Ultrafast liquid chromatography; **FDA:** Food and drug administration; **GC-MS:** Gas chromatography mass spectroscopy; **IR:** Infrared Spectroscopy; **HS-SPME:** Headspace-solid phase microextraction.

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