

Mini Review: Chemical Characterization and Evaluation of Antiviral and Antibacterial Activity of Extracts from *Graptopetalum paraguayense* E. Walther (*Crassulaceae*)

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Abstract

Graptopetalum paraguayense E. Walther (GP) is a traditional Chinese herbal medicine belonging to the *Crassulaceae* family. As herbal medicine, GP possesses several biological/pharmacological perspectives. It was found that GP aqueous extracts exhibit anti-inflammatory and hepatoprotective, antioxidant, anti-hypertensive and antihyperglycemic, immunomodulatory, antineoplastic, antiviral and antibacterial activities. The GP contains in the high range levels phenolic compounds and anthocyanins. Despite the intense study of the plant, little data exist regarding its chemical composition. It was found that the GP aqueous extract has no cytotoxic effect on RD and Lep cells. The extract effectively inhibited herpes simplex virus (HSV) replication in dose-dependent manner moderate activity as well as Gram-positive bacterial strains.

Keywords: *Graptopetalum paraguayense* E. Walther (GP); pharmacological perspectives; anti-herpes simplex virus effect; cell culture; toxicity.

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1. Introduction

Throughout our evolution, the importance of natural products for medicine and health has been enormous. Owing to the diverse biological activities and medicinal potentials of natural products, nearly every civilization has accumulated experience and knowledge of their use. The oldest medical text comes from ancient Mesopotamia, circa 2600 BC, later in the Orient and Occident.

Modern chemistry has ushered in a new era for the study and use of natural products. Analytical and structural chemistry have provided the tools to purify various compounds and to determine their structures, which, in turn, has given insights into their action on the human body. The structural analysis of natural compounds and the ability to synthesize them allowed chemists to modify them in order to suppress or enhance certain characteristics such as solubility, efficiency or stability in the human body [1].

Plants are the oldest source of pharmacologically active compounds, and have provided humankind with many medically useful compounds for centuries. It is possible that antimicrobial compounds from plants may inhibit bacteria by a different mechanism than the presently used antibiotics and may have clinical value in the treatment of resistant microbial strains. For this reason, it is therefore important to investigate plants as alternative sources of anti-microbial compounds. Plant-based medicines, unlike pharmaceuticals, don't cause resistance problems, they are much safer, and they are ecologically sound — they are biodegradable and renewable, which most pharmaceuticals are not [2]. As herbal antibiotics, antiviral herbs are easy to use, easy to grow, and easy to make into medicines. They are very effective for emerging and resistant viral infections [3].

2. Natural products - chemical content and activity

Due to the increase of antibiotics resistance, there is an urgent need to develop new and innovative antimicrobial agents. Plants have long been investigated among the potential sources of new agents. They contain many bioactive compounds that can be of interest in therapy. Because of their low toxicity, there is a long practice of using dietary plants in the treatment of infectious disease in the world's traditional medicine [4].

Plants have shown considerable activity against various microbes [5-8]. This activity is due to secondary metabolites which are found in several plant tissues [9]. These secondary metabolites could be phenols, saponins, quinolones, flavones, flavonoids, flavonols, tanins, terpenoids, essential oils, alkaloids, polypeptides and other compounds [10], and in spite that at least 12,000 secondary metabolites have been isolated, it is believed that they represent less than 10% of the total of the metabolites that really exist [11]. These substances could be responsible for the defence mechanisms of the plants against damages caused by microorganisms, insects and other animals [10]. Plant extracts are known to consist of many chemicals and among them, a few compounds could be acting synergistically. Sometimes, isolation of the compounds from the extract may cause a decrease in desired activity, which underlines the importance of extract screening. It is considered that plants are a source of a wide variety of bioactive molecules that can be used for the development of new medicines with a wider spectrum of activities and with less adverse effects than those produced by the drugs currently in use.

The bioactive compounds as flavonoids, alkaloids, saponins, tannins, quinones, and sterols/triterpenoids have

been reported to be used by plants for protection against bacterial infections and are responsible for antimicrobial activity. Plant extracts and plant-derived compounds represent an important source of new potential antiherpetic drugs [6,7]. Phenolic compounds are of great interest due to their antioxidant activity and beneficial effects on human health. The results of a preliminary tests of green solvent extract (hydroethanol) shoots of *Limonium densiflorum* and acetone and methanolic apple cider extract show that phenolic compounds, in particularly gallic acid and epigallocatechin gallate have strong anti-herpes activity [9] and inhibit both HSV-1 and HSV-2 replication in Vero cells by more than 50% at non-cytotoxic concentrations [10]. Flavonoids are polyphenolic compounds present throughout the plant kingdom and capable of interacting with many biological systems, including Herpes simplex virus (HSV) [8-10]. Studies on the antiherpetic activity of flavonoids mainly focus on widespread substances such as flavones, flavonols and chalcones [8,11,12].

Infectious diseases are the leading cause of global morbidity and mortality. 90% of infectious diseases in humans have viral etiology. Frequent human infections of viral origin could be exemplified as common cold, influenza, varicella, AIDS, herpes simplex, infectious mononucleosis, avian influenza etc.

So far, eight herpes viruses that attack humans have been detected. These are: Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), varicella zoster/herpes zoster virus (VZV/HZV), human cytomegalovirus (HCMV), Epstein-Barr Virus (EBV) and human herpes viruses 6, 7 and 8 (HHV-6, HHV-7 and HHV-8).

Mononucleosis is caused by the Epstein-Barr virus (EBV). EBV is a member of the herpes virus family and is one of the most common viruses to infect humans around the world. EBV is associated with malignant tumors, and in particular EBV is associated with Burkitt's lymphoma [12], a B-cell derived childhood malignancy that is endemic in the rain forest regions (malarial areas) of equatorial Africa. Elsewhere in the world the association with EBV is less consistent ranging from 20% to 80%.

Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) are members of the *Herpesviridae* family and are among the most common human pathogens, infecting about 90% of the world population [13]. HSV-1 and HSV-2 are usually associated with orolabial and genital lesions, respectively [14]. HSV can also cause deadly infections of the central nervous system and disseminated disease in immune-compromised adults and newborns [14, 15]. Antiviral therapies for HSV are only partially effective since the virus can ascend along neuronal axons to establish latent infections that last a lifetime [13, 16]. Because viruses use the host cell to reproduce and reside, it is rather difficult to exterminate them without killing the host cell. The most effective treatment strategies for viral diseases are vaccination, which provides resistance to infections, as well as antiviral drugs, such as nucleoside analogues (acyclovir, ribavirin, penciclovir, valacyclovir and famciclovir).

In recent years, the significance of herpes infections has increased as a parallel disease and as a factor in an aggravated clinical picture in AIDS patients. In many cases, the lethal outcome in these patients is a result of herpetic relapse. The treatment of HIV/AIDS by the introduction of antiretroviral therapy (ART, HAART) is considered to be one of the greatest successes of modern medicine. Application of antiviral therapies to patients is often difficult because of drug toxicity, where the successful application of HAART is difficult and conditions for the emergence of mutants with multiple resistance. Despite advances in the influenza viruses science, they

are currently one of the most widespread viruses of major socio-economic importance. Important human and animal pathogens are included in the seven genera of the *Orthomyxoviridae* family. Influenza A, B and C viruses (*Influenza virus* A, B and C) are of significant importance to humans. Almost all of the flu viruses circulating in recent years are resistant to ion channel inhibitors *M2*, which makes ineffective the use of adamantans (the main group medicines against influenza) for the treatment and prevention of influenza infections. Therefore, it is imperative to seek and detect new antiviral substances against both known and alternative targets that occur during the life cycle of the virus. For this reason, scientists' attention is directed to some new compounds of natural origin, as well as to new untapped viral life cycle targets. Many small molecules such as phenols, polyphenols, terpenes, flavonoids, sugar-containing components that have been shown to be promising antiviral agents have been proven in plant extracts.

The main causes of the most common infectious diseases in humans belong to two groups - Gram-positive and Gram-negative. The bacteria *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Bacillus cereus* belong to the first group, while *E. coli*, *Pseudomonas aeruginosa* and *Salmonella Dublin* are Gram-negative bacteria.

Global prevalence of infectious diseases caused by bacteria is a major public health problem. The bacterial agents including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* cause several human infections [17]. *Staphylococcus aureus* and *Escherichia coli* are a major cause of various humans and animal's infections. The first causes skin and soft tissues infections, surgical site infections, and bone and joint infections. *Staphylococcus aureus* is a common cause of hospital-acquired bacteraemia and it is associated with hospital-acquired respiratory tract infections. *E. coli* is the most common cause of urinary tract infections (UTIs) in humans, and is a leading cause of enteric infections and systemic infections. The systemic infections include bacteraemia, nosocomial pneumonia, cholecystitis, cholangitis, peritonitis, cellulitis, osteomyelitis, and infectious arthritis and neonatal meningitis [18].

Streptococcus pyogenes, a gram-positive facultative anaerobe and the etiological agent can cause a variety of diseases including streptococcal pharyngitis, impetigo and rheumatic heart disease, dependant on which tissue it infects. *Bacillus cereus* causes two different types of food poisoning: the diarrhoeal type and the emetic type. *Pseudomonas aeruginosa* is a multidrug-resistant pathogen, recognized early for its ubiquity, and its mechanism of advanced inherently antibiotic resistance. As an opportunistic human pathogen, *P. aeruginosa* is a common cause of nosocomial infections and is responsible for persistent infections in immunocompromised individuals and for the chronic lung infections of patients with cystic fibrosis [19]. However, due to the selflimiting nature of these infections and the increasing risk of antibiotic resistance unless complications arise monitoring and symptom treatment is often the preferred option. The probing of natural plant resources offers an alternate means of fighting bacterial diseases through the prevention of bacterial growth [20].

3. Graptopetalum paraguayense E. Walther (Crassulaceae) - medicine and pharmacology applications

Family *Crassulaceae* or orpine family, stonecrop family, Synonym: *Sedaceae*, is widely distributed for horticulture and it is a large family of dicotyledons, consisting mainly of succulent herbs, but tending to be

miniature shrubs or trees in certain genera and species. Most members of the family are remarkable for their xeromorphic structure, particularly the occurrence of water storage tissue in the leaf and stem. Some are believed to be capable of absorbing water directly from the air by special hairs, epidermal cells or adventitious roots. The family includes about 30 genera and the *Graptopetalum (leatherpetal)* is a plant genus of this family. It is known that some members of the *Crassulaceae* family exhibit antiseptic and antibacterial properties [21]. There are a reports [3, 22, 23] that the n-hexane fraction, the ethyl acetate soluble fraction and the crude leaf extract of *Bryophyllum pinnatum* S.(*Crassulaceae*) showed antibacterial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Escherichia coli*. The juice from the 4 species belonging to the genera *Kalanchoe (Crassulaceae)* had shown high virus neutralizing activity [24, 25]. Antimicrobial activity was reported for *K. daigremontiana* extracts, without identification of the responsible bioactive compounds [26, 27]. Several butadienolides and phenolic substances were isolated from this plant [28, 29]. However, the only flavonoid reported for *K. daigremontiana* is kaempferol-3-*p* -coumaroylarabinoside, named bryophylloside [23, 24]. The presence of bioactive flavonoids in other *Kalanchoe* species was reported in some studies [15]. The lipophilic extract of *Sedum hispanicum* L. (*Crassulaceae*) showed a very high inhibition against HSV-1 type a comparably close to that of acyclovir [30].

Graptopetalum paraguayense E. Walther (GP) (Figure 1) is a species of succulent plant in the jade plant family, *Crassulaceae*. It is a succulent, drought-resistant perennial grown as ornamental houseplant in temperate regions, as it cannot survive winter outside. The plant is native to Mexico and is distributed widely in tropical and subtropical countries where it is mainly cultivated as ornamental plants, but are popular in Chinese herbal medicine. It is a widely consumed plant food in Taiwan and it is used in folk medicine. This herb possesses several health benefits. According to its archaic Chinese prescription, GP is able to alleviate hepatic disorders, lower blood pressure, whiten skin, relieve pain, treat infections, inhibit inflammation, acts as a diuretic and improve brain function.



Figure 1: *Graptopetalum paraguayense* E. Walther (*Crassulaceae*).

GP and its applications were intensively investigated especially in the last years. Recently, the anti-inflammatory effects of water extracts of GP in subjects with metabolic syndrome was investigated [31].The in vivo study of mammalian erythrocyte micronuclei confirmed that GP aqueous extractis safe and has no mutagenicity [32]. It was found that GP contains various potential antioxidants, which may help reduce oxidative damages that occur in the human body and prevent lipid peroxidation in foods. In addition to the

antioxidative activity of *Graptopetalum paraguayense*, several studies have also demonstrated that GP has various biological properties, including inhibition of mushroom tyrosinase, angiotensin-converting enzyme activity, and production of an antimutagenic effect [33-35] (Figure 2). The article [36] is reported that hot-water extract from GP leaves shows very promising antioxidant activities, anti-colon cancer activity (colon adenocarcinoma Caco-2 cells) and anti-inflammatory activity in brain neuro cells. The results in the paper [37] demonstrated that *Graptopetalum paraguayense* E. Walther ethanolic extracts clearly inhibited airway inflammation, mucus cell hyperplasia, and eosinophilia in OVA-challenged mice. Lee et al. [38, 39] investigated the protective properties of GP 95% ethanol extracts against Carboxymethyllysine (CML)-induced pancreatic damage. GP extract and gallic acid (the active compound of GP extract) could potentially be used as a food supplement to protect against pancreatic damage and the development of diabetes. There are a data that suggest the effects of *Graptopetalum paraguayense* E. Walther against inflammatory liver damages, protection against

hepatic fibrosis in hepatitis C and liver cancer in rats [40-44]. A reported patent [45] depicted that GP is potent for the protection of animal liver diseases and medical conditions, such as inflammation, steatosis, and fibrosis. In particular, *Graptopetalum paraguayense* inhibits proliferation of activated hepatic stellate cells, which play a pivotal role in liver fibrosis. Furthermore, the plant is useful against fibrosis or inflammation of tissues or organs other than the liver, in particular lung, kidney, and bladder. They mentioned in the patent that clinical trial results indicate almost all patients (14 persons) completely recovered from hepatic steatosis, thus, GP is effective in patients with fatty liver. A previous study has also indicated that an ethyl ether extract of GP possesses neuroprotective and anti-inflammatory activities regarding inflammation-associated neurological diseases [46]. The plant has been predicted to be a potential therapeutic agent for Alzheimer's disease from among Chinese herbal medicines (CHMs), by using gene set enrichment analysis (GSEA). The findings suggests moving extracts of GP toward drug development, either for treating of this disease or as a health supplement to prevent Alzheimer's disease [47].

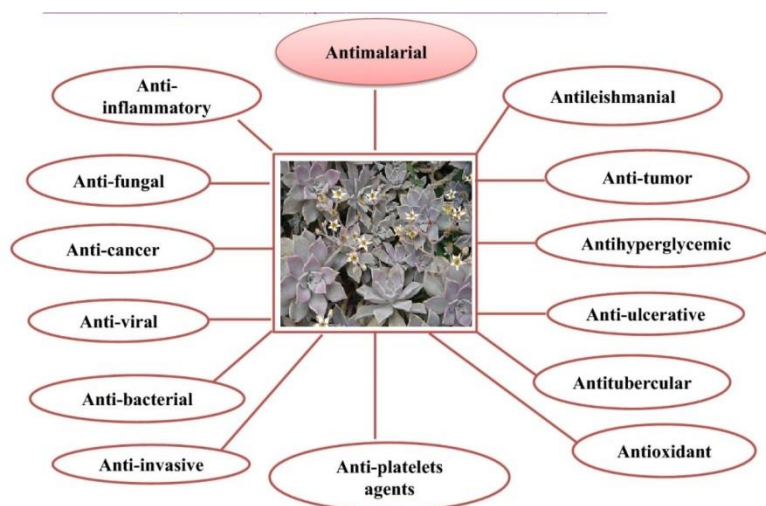


Figure 2: Applications of *Graptopetalum paraguayense* E. Walther in medicine and pharmacology.

Despite the intense study of the plant, little data exist regarding its chemical composition. Liu et al. [48] isolated four major components from its MeOH extract and determined their structures to be (acetylated) HMG-substituted flavonol glycosides, which are rare in nature. These compounds as well as gallic acid appear to be responsible for the antioxidant activity of GP extracts [49].

However, there is no information in the literature on the anti-conjunctivitis, antiviral and antibacterial activity of *Graptopetalum paraguayense* E. Walther.

Part of the authors of this article was published a preliminary study on the chemical composition and anti-conjunctivitis effect of GP leaf fresh juice [50]. Fresh juice of *Graptopetalum paraguayense* leaves applied directly to the irritated eye two times per day cured conjunctivitis in all reported cases. The main groups of organic compounds identified by GC-MS analysis in the fresh extracted leaf juice of *Graptopetalum paraguayense* were: alkylamines, hydroxycarboxylic acids, aliphatic and aromatic carboxylic acids, amino acids, alcohols, aromatic and aliphatic hydrocarbons. It is suggested that GP exerts anti-conjunctivitis activity, through synergistic effect of different chemical compounds, most probably alkylamines and mainly hydroxycarboxylic, aliphatic, and aromatic carboxylic acids.

A preliminary investigation on antiviral and antibacterial activities of GP extracts was performed by our team [51]. It was found that the GP aqueous extract has no cytotoxic effect on RD and Lep cells. The extract effectively inhibited HSV replication in dose-dependent manner moderate activity as well as Gram-positive bacterial strains. Furthermore, the aqueous extract was more effective inhibitor of HSV-1 replication in cultured cells, as their IC_{50} values were not so significantly lower than that of ACV. It was inhibited the HSV-1, Victoria strain replication 97%, whereas its effect to HSV-2, strain Bja was significantly lower. It was found that the GP aqueous extract has no cytotoxic effect on RD and Lep cells. The extract effectively inhibited HSV replication in dose-dependent manner. Furthermore, the aqueous extract was more effective inhibitor of HSV-1 replication in cultured cells, as their IC_{50} values were not so significantly lower than that of ACV. It was inhibited the HSV-1, Victoria strain replication 97%, whereas its effect to HSV-2, strain Bja was significantly lower. The tested GP extract was 20 times more effective and selective as an inhibitor of the growth of the ACV^R mutant DD (HSV-1) compared to ACV and gave cell protection of 65.5%. The selectivity of the antiviral action to GP extract was confirmed by the variation in sensitivity of different HSV-1 and HSV-2 strains sensitive or resistant to ACV. The different results for the inhibitory effects against HSV strains may be due to some differences in replication, regulatory proteins (ICP group) and presence of mutations in the genes coding for some viral enzymes (especially thymidine kinase (TK)). The antiviral efficacy of ACV is related to the specific inhibition of the viral DNA polymerase (DNApol) during the replication cycle when new viral DNA is synthesized [52].

To clarify what causes this inhibitory activity on HSV-1, a metabolic profile of *Graptopetalum paraguayense* was performed and a voucher specimen (SO 107 621) has been deposited at the herbarium collection of Sofia University. Three main fractions: non-polar substances (fatty acids, sterols and terpenoids), polar metabolites (aminoacids, hydroxycarboxylic acids, sugars, and sugar alcohols) and phenolic compounds (gallic, *trans*-ferulic, syringic, gentisic, vanillic acids and others) were obtained and GC-MS analysis was carried out (Table1) [53]. Since it is well known that phenolic compounds show a significant anti-herpes effect and that viral DNA

polymerase appears to play a key role in HSV virus replication, we have presented a docking and quantum-chemical analysis of the binding of these compounds to viral DNAPol amino acids [53]. Fourteen different phenolic acids found by GS/MS analyses (Table1), were used in molecular docking simulations. According to the interaction energies of all fourteen ligands in the DNAPol pockets based on docking results, DFT calculations at B3LYP/6-31+G(d,p) computational level were performed on the five optimally interacting with the receptor acids to establish the binding affinity of hydroxybenzoic acids from GP to HSV DNAPol active site.

Table 1: Phenolic components fraction from *G. Paraguayense* determined by GC-MS analysis

#	Name	RT	RI _{lit}	RI _{calc}	µg/g DW
1	Salicylic acid (2TMS)	8.01	1510.2	1510.3	41.57
2	<i>m</i> -Hydroxybenzoic acid (2TMS)	8.51	1576.9	1577.0	24.68
3	4-Hydroxyphenylethanol (2TMS)	8.58	1580.6	1580.7	18.35
4	<i>p</i> -Hydroxybenzoic acid (2TMS)	9.19	1640.3	1640.4	20.32
5	<i>p</i> -Hydroxyphenylacetic acid (2TMS)	9.34	1665.8	1665.9	32.98
6	Phloretic acid (2TMS)	10.89	1763.2	1763.3	58.94
7	Vanillic acid (2TMS)	11.02	1776.0	1776.3	80.32
8	Gentisic acid (3TMS)	11.15	1788.8	1788.9	70.59
9	Protocatechuic acid (3TMS)	11.77	1813.3	1813.4	85.50
10	Syringic acid (2TMS)	12.92	1911.3	1911.4	291.36
11	<i>p</i> -Coumaric acid (2TMS)	13.48	1946.9	1947.0	147.80
12	Gallic acid (4TMS)	13.92	1969.0	1969.1	183.27
13	<i>trans</i> -Ferulic acid (2TMS)	15.90	2103.0	2103.1	218.97
14	1,8-Dihydroxyanthraquinone	16.25	2125.5	2125.6	426.84
Total phenolic compounds					1701.50

DW - dried weight, **RT** - retention time, **RI** - Kovats retention indices, **TMS** - trimethylsilyl derivatives

According to the interaction energies of all five ligand-amino acid complexes, the hydrogen bonding in all of them is strong but significantly weaker than that in the acyclovir complex. The reason could be in DFT methods used as well as in the mechanism of antiviral effect. It is possible the mode of the inhibitory activity on the virus replication of acyclovir triphosphate (by DNA polymerase) is different from that of the plant hydroxybenzoic acids one. Compared to ACV (with total protection of the cells 100%), the anti-HSV-1 effect of the GP extract was less pronounced (97.5% cell protection) but due to its different nature, viral mechanism of action and low cytotoxicity, it has the potential to be used as an additive in the treatment of HSV-1 infections after detailed

evaluation of its pharmacological interactions with clinically applied anti-herpetic drugs.

Acknowledgements

This study was supported by the Bulgarian National Science Fund under Grant No DN 19/16/2017.

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