Iranian Reasearch Organization
for Science and Technology
(IROST)

Advances in Environmental Technology 9(4) 2023, 271-293

Advances in Environmental Technology

Journal home page: https://aet.irost.ir/

A review on water disinfection with plant products-

Wali Humayun[*], **Ahmad Muhammad Mahmood**

Chemical Engineering Department University of Engineering and Technology, Lahore

ARTICLE INFO

Document Type:
Review Paper

Article history:
Received 2 September 2022
Received in revised form
26 July 2023
Accepted 30 July 2023

Keywords:
Water disinfection
Herb extract
Phytochemical
Metal complex

ABSTRACT

Background

Conventional techniques for water disinfection are fraught with issues like personnel exposure to damaging radiation and formation of harmful and carcinogenic disinfection byproducts. There are difficulties related to transportation and handling, and expensive capital and working costs also are involved like costs associated with on-site generation of disinfectants. There is a dire need for newer disinfection technologies that are environment and health friendly.

Scope and benefits

This article reviews the use of natural disinfectants derived from plants to enhance the quality of water. Researchers have utilized herbal extracts, phytochemicals, and phytochemical-metal complexes for the disinfection of water. Various factors for these chemicals like efficacy, toxicity, cost, and water solubility have been discussed and some useful phytochemical disinfectants are also identified. These disinfection methods particularly when using only pure phytochemicals are generally thought to be free from the deleterious effects associated with chlorination and other conventional technologies. Inherently, chlorinated and other harmful disinfection byproducts are not formed.

Key findings and conclusions

In various studies eugenol, thymol and extracts of *Ocimum sanctum* and *Azadirachta indica* have been utilized with fairly effective disinfection capabilities. The significant antimicrobial effects of allicin, berteroin, sanguinarine, and thymol are reflected from their very low minimum inhibitory concentration values. Even so, presently the efficiency of phytochemicals is not comparable to conventional disinfectants. The use of phytochemical metal complexes is, however, a plausible option that might be investigated further. The metal complexes because of their greater water solubility than pure phytochemicals result in improved disinfection efficiency. Notable among those are flavonoid-metal complexes that should be considered further for use in water disinfection. It is also concluded that phytochemicals may be added to water that has also been disinfected with some other commonly-used

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[*] Corresponding author Tel.: 923350477583
E-mail: hwali@uet.edu.pk

DOI:10.22104/AET.2023.5821.1605

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technology. A way to do this may be to design a fixed bed tower of phyto-disinfectant through which water should pass.

Contents

1. Introduction

Water is an essential element for human survival. However, it is being polluted by various environmental contaminants. According to World Health Organization statistics, globally at least 2 billion people use a drinking water source contaminated with microorganisms, and such water is estimated to cause 485,000 diarrheal deaths each year [1]. The areas that are worst hit in this regard are villages and small settlements. The main reason for this is the lack of facilities for purification of drinking water. Ground and surface waters can be made potable and palatable by adequate treatment steps. Disinfection is the most important step to ensure that water is free of harmful microorganisms. Many technologies have evolved at commercial and industrial levels resulting in the use of several disinfectants that are currently available in the market [2]. Chemical methods (chlorine and its derivatives, ozone etc.), and physical methods (UV radiation, ultrasonication, etc.) can be regarded here as conventional disinfection methods. However, there are issues related to their use which cannot be discounted. Chemical disinfectants, for example chlorine and ozone, are usually very toxic and harmful to the environment. Physical disinfectants, such as UV radiation and ultrasonication, can be dangerous on exposure. Conventional methods may also require special handling and transportation (e.g., chlorine), or on-site generation (e.g., ozone, UV radiation). Another hazardous aspect is the formation of disinfection byproducts (DBPs) chiefly from chlorine-based disinfectants. Other disinfectants such as ozone and UV radiation also produce their own byproducts [3]. The DBPs are thought to have been linked with serious health issues e.g., cancer, and reproductive disorders [4]. If a chemical disinfectant is used, water needs to be purified thoroughly before disinfection to remove the natural organic matter (NOM), which is a precursor to the DBPs. Additionally, there are taste and odor issues related to conventional disinfectants, especially with the chlorine-based disinfectants. Among other alternatives, medicinal and aromatic plants can be used for making freshwater potable. In addition to basic functions, plants possess certain chemicals that have secondary functions such as defense against pathogens, predators, etc. These chemicals in plants are formed due to secondary metabolism and are categorized as phytochemicals. These have many additional functions such as antioxidant properties and potential to kill or at least immobilize microbes [5]. There are many ways in which plants may be utilized for the treatment of drinking water. Herbal extracts along with a solvent [6], herbal extracts in the presence of metallic salts [7,8], metal complex of a phytochemical [9-11], and herbal extracts combined with established disinfection technology [12] have been used. It is generally believed that these methods provide a greener way to purify water as compared to conventional chlorination and related disinfection techniques. In addition to this, metal complexes of phytochemicals have been associated with detoxification of the metal cations in humans, animals and plants [13]. The use of plants as antimicrobial products is an ancient practice. Capasso [14] has reported that Ötzi the Iceman, who lived about 5300 years ago, had berries, two birch-bark baskets, and two species of polypore mushrooms with leather strings through them in his possession. The birch fungus is known to have anthelmintic properties and was probably used by Ötzi as an antimicrobial agent.

2.Methodology of data collection and processing

This article reviewed the attempts made by various researchers who utilized plants, phytochemicals, and phytochemical-metal complexes for disinfection of drinking water. A thorough search of relevant original research literature was carried out to identify nearly 150 herbs and 100 phytochemicals that have been or could be utilized for disinfecting water from planktonic as well as sessile microorganisms. Various databases were also consulted to validate their antimicrobial characteristics, metal complexing ability and other pertinent properties. They also provide information on herbs as well as phytochemicals. Examples of these used here are referenced in [15-17]. The antimicrobial activities were also checked from PASS Online [18] software. The cost data was gathered from different (undisclosed) vendors and actual costs are not reported but a number is allocated for the same unit mass (or volume) of each phytochemical to give an idea of relative costs between different phytochemicals. Thus a $ 100/25 mg is taken as 1 unit of cost and $ 100/25 mL is also taken as 1 unit of cost. For example, on this scale, $ 200/25 mg is 2 units, and $ 100/100 mg is 0.25 units. A web resource with data on toxicity of chemicals is the NLM (National Library of Medicine) database, URL: https://www.nlm.nih.gov. Data was gathered from this database and its sub-databases for antimicrobial phytochemicals of interest. This database is useful in the context of describing the human toxicity values obtained from numerous clinical studies and peer reviewed literature. The GUSAR Online [19] software was used to verify toxicity of phytochemicals.

3.Target species of microorganisms

While a considerable literature exists on action of a herbal extract or a phytochemical on one kind of microorganism such as reported by [20-22], work on some general group of microorganisms (e.g. coliforms) commonly found in drinking water is rarely reported. Therefore, in the context of disinfection of drinking water, it is important to have knowledge of the kinds of microorganisms present in water. *E. coli* is an effective indicator of fecal contamination in drinking water [4]. Whereas the groups 'Coliforms' and 'Fecal Coliforms' are also present in water [23], *Enterobacteriaceae*, *Streptococcus*, *Enterococcus*, and *Listeria* are among the commonly found genus of bacteria in water. Apart from this, many fungi, protozoans, and viruses are also commonly found in water [23-25]. Some of these like coliphages may not be a serious hazard, but protozoans like *Giardia lamblia*, *Cryptosporidium parvum*, *Naegleria fowleri*, and viruses like polio and hepatitis pose a grave health concern. The bacteria which are present in human gut present an interesting study. Consequently, many of the plants and phytochemicals mentioned in this review have also some antimicrobial effects on the gut bacteria. Many diseases that are caused by unhygienic water affect the gastro-intestinal tract [1]. In the discussion that follows it can be seen that many of these plants are also used by hakims (apothecaries). It reiterates the fact that hakims gainfully advocate the use of these herbs in gastro-intestinal ailments.

4. Plants utilized for water disinfection

A variety of species from the plant kingdom have been utilized by researchers for water disinfection. As these plants are identified, it becomes easier to find the phytochemicals that may have played useful role in disinfection of water. Lutgen and Michels [26], worked on bactericidal effect of *Artemisia annua* (argy wormwood). Adding 50% of *Artemisia annua* tea had a stronger bactericidal effect than boiling contaminated water (5 min) or irradiating (10 min) under 365 nm UV radiation. It was found that when tea was freshly prepared, artemisinin and scopoletin exerted the microbicidal effect. *Coridothymus capitatus* (Mediterranean wild thyme), had been discussed by Winward et al. [27], for its disinfection efficacy of grey water. It was found that the origanum oil from the plant and carvacrol exerted significant antimicrobial activity. At 468 mg/L, in 100 mL grey water, zero percent total coliforms were detected. An area equal to 35 UK average households was calculated to produce enough origanum oil for disinfection of grey water as used in toilet flushing. Ahmed et al. [28], studied aqueous and methanolic extracts of *Colebrookia oppositifolia* (Indian squirrel tail). It was discovered that the root extract was more effective against waterborne pathogens than the fruit extract. Shaheed et al. [29], applied the fruit and seed extracts of *Luffa cylindrica* (dishrag gourd) in water to monitor reduction in total and fecal coliforms. A maximum of 86% inactivation of coliforms was documented, and the seed extracts were found to be better disinfectants than the fruit extracts. Harding and Schwab [30] experimented with lime (juice and extract) and psoralens in combination with solar disinfection of water. More than 6.1 log reduction for *E. coli*, and more than 3.9 log reduction for MS2 bacteriophage was recorded with a combination of lime slurry and solar disinfection. It was concluded that a synergistic relationship should be present between low pH water and solar radiation for water disinfection. Harikumar [31] studied the potential of herbal extracts of three *Ocimum* spp., *Azadirachta indica*, *Simarouba glauca*, *Caesalpinia sappan*, *Cuminum cyminum*, *Vetiveria zizanioides*, *Saraca indica*, and *Murraya Koenigii*, against different bacteria such as total and fecal coliforms, *E. coli*, *Bacillus* sp. and *Serratia* sp. for utilization in water disinfection. *Ocimum sanctum* could be most effectively used in water treatment applications. Eugenol and β-caryophyllene (major constituents in the essential oil of *Ocimum sanctum*), were deduced to be responsible for its antimicrobial activity. Ramavandi [32], successfully extracted a bio-coagulant from *Plantago ovata* using $FeCl_3$-induced crude extract. In addition to lowering the water turbidity, significant improvement in bacteriological quality of water was also observed. At a very low concentration of 0.25 mg/L of the extract, significant coagulation was achieved. Pandit and Kumar [33] reviewed various methods for disinfecting water feasible for the developing countries including the use of herbs in this context. Kingsely et al. [34], tested *Garcinia kola* (bitter kola) and *Carica papaya* (papaya) seeds mixture extract for biodisinfection and coagulation of water. Antimicrobial efficiencies of up to 83.3% for total coliforms and up to 74.4% for heterotrophic bacteria were reported. These nontoxic seeds could possibly be used for water treatment. Extracts of seven Moroccan plants were tested against *E. coli* by Douhri et al. [35]. The ethanolic extract of *Origanum elongatum* (oregano) leaves was observed to be most effective with an inhibition zone of 30.3 mm diameter. *Myrtus communis* (common myrtle) leaves exhibited 20.2 mm and *Punica granatum* (pomegranate) seeds exhibited 17.3 mm zones of inhibition. It was established that a strong correlation exists between antibacterial activity and the percentage of phenolic compounds in the extracts. Dhivya and Kalaichelvi [36], extracted the bioactive compounds from *Sarcostemma brevistigma* and identified that 3,4[methylenedioxy] phenethylamine, 1-(dimethylamino)-4,5-dihydro-3-methyl-1H-benz[g] indole, and 2,9-bis[(diethoxyphosphinyl) methyl]-1,10-phenanthroline possessed drinking water disinfection capability. Okunlola et al. [37]used citrus spp. on pond water and studied its phytodisinfectant properties. The stem of *Citrus aurantifolia* after 12 h of use completely eliminated the total viable count, total coliform count and fecal coliform count. The presence of various phytochemicals was attributed to the disinfection capability, but none was preferred. Adeeyo et al. [38] experimented with extracts of *Zanthoxylum zanthoxyloides* and *Gongronema latifolium* on waterborne bacteria and fungi. At 500 mg/mL the ethyl acetate extract of *Zanthoxylum zanthoxyloides* resulted in up to 27 mm zone of inhibition against *E. coli*, whereas the chloroform extract of the same herb produced a 20.5 mm zone of inhibition against the *Trichoderma* sp. Adeeyo et al. [6] thoroughly reviewed the utilization of plants in water potabilization and disinfection. They established that more research had been done regarding the antimicrobial activities of phytochemicals than their utilization in water treatment. Many challenges that needed to be resolved were also discussed, including the complex nature of plant extracts, lack of their standardization, poor water solubility and their extraction and purification complexities. *Moringa oleifera* (horse radish tree) has been extensively studied for removal of water turbidity and disinfection. Ahmed et al. [39], experimented on the methanolic and aqueous extracts of *M. oleifera* on a few pathogenic spp. The buds and shoots extracts were more antibacterial against different bacteria than seeds and leaf extracts at 37 °C. Seeds had the highest coagulation activity, higher even than alum. Adejumo et al. [40], worked on the dried leaves powder of *M. oleifera* on *E. coli* and other pathogenic bacteria. A narrow spectrum of antibacterial activity was found with no significant reduction in coliform count and no antibacterial activity against *E. coli*. Yongabi [41], described a 'phytodisinfectant' based sand filter. Extracts of *Moringa* seed powder in various solvents exhibited up to 85% antibacterial activity against *E. coli* and 95% against *Aeromonas hydrophila*. Yongabi et al. [42], researched on the aqueous and methanolic extracts of seeds powders of *M. oleifera*, *Garcinia kola*, *Jatropha curcas* (physic nut), *Carica papaya*, *Persea americana* (avocado), and *Hibiscus sabdariffa* (roselle), which they tested on different types of wastewater. Remarkable results were found, for instance, untreated stormwater that had a total heterotrophic plate count as too numerous to count, resulted in the largest decrease in case of *M. oleifera* seeds to 120, followed by *Carica papaya* at 398; their target microorganisms were heterotrophic plate count, coliforms, and *E. coli*. Lea [43], developed a protocol regarding the use of *M. oleifera* seeds for coagulation and subsequent disinfection of turbid water. Up to 99.5% reduction of turbidity was achieved accompanied by up to 4 log bacterial reduction. In another example, the seeds of *M. oleifera*, have been used to clarify turbid water by Baptista et al. [44]. Similarly, the natural coagulant of *M. oleifera* has been successfully used to remove turbidity by Keogh [45], prior to solar disinfection (SODIS) of model *E. coli* in water. Antimicrobial activity of *M. oleifera* seed powder was studied by Luwesi et al. [46]. Zones of inhibition were measured using surface waters, and were found as follows: 21 mm for *S. aureus*, 16 mm for *Salmonella typhi*, and 10 mm for *E. coli*. Wali [47] selected 37 herbs with antimicrobial activity which could potentially be used for disinfection of water. These are presented in Table 1. In the Kirby-Bauer Anti-microbial Susceptibility Test, 0.08 g/mL methanolic extract of *Polygonum viviparum* exerted considerable antimicrobial activity against the environmental thermotolerant HPC bacteria and had an inhibition zone of 9.7 mm.

4.1. Traditional antimicrobial use of plants

Historically, different civilizations have used plants to cure various diseases. Leporatti [48] described a few such plants. The more significant ones are as presented in Table 2.

Many of the diseases related to gastro-intestinal system are caused by drinking unhygienic water [1]. If it could be identified as to how many times a plant species is utilized as an ingredient in medicines, some knowledge might be gained on their efficacy as antimicrobial agents against bacteria residing in the gastro-intestinal tract. A few such plant species have been listed in Figure 1.

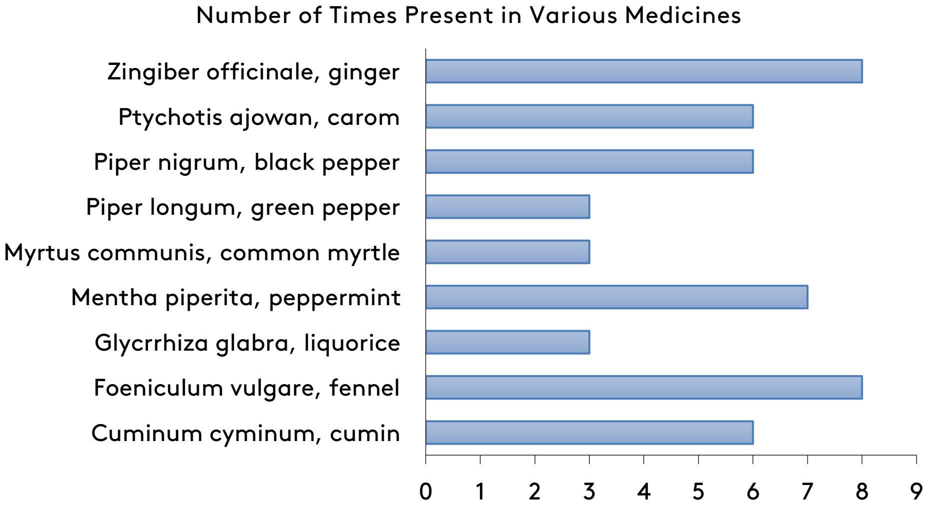

Fig. 1. Common ingredients and the number of times they were noticed in different medicines of gastro-intestinal system.

Table 1. Herbs that should be experimented for water disinfection capability.

S #	Botanical Name	English Name
1	*Achillea millefolium*	Yarrow
2	*Amomum subulatum*	Black cardamom
3	*Anethum graveolens*	Dill
4	*Artemisia annua / absinthium*	Sweet wormwood, Annual mugwort / wormwood
5	*Caryophyllus aromaticus*	Clove
6	*Cassia acutifolia*	Alexandrian senna
7	*Colebrookea oppositifolia*	Indian squirrel tail
8	*Commiphora opobalsamum*	Balsam of Gilead, Arabian balsam tree
9	*Coridothymus capitatus*	Conehead thyme, Mediterranean wild thyme, headed savory
10	*Cuminum cyminum*	Cumin
11	*Curcuma longa*	Turmeric
12	*Curtisia dentata*	Assegai tree
13	*Cuscuta reflexa Roxb*	Dodder (Cuscuta spp.)
14	*Cyperus articulatus (Cyperus pertenuis)*	A sweet-smelling grass. Papyrus sedges (Cyperus spp.)
15	*Echinacea purpurea*	Purple coneflower
16	*Erythrina zeyheri*	Harrow breaker, plough breaker
17	*Foeniculum vulgare*	Fennel
18	*Hardwickia binata*	Hardwickia
19	*Huernia hystrix*	Toad plant
20	*Hydrastis canadensis*	Goldenseal, orangeroot
21	*Hymenaea stigonocarpa*	Jatob'a do cerrad'o
22	*Hypericum perforatum*	St. John's wort
23	*Ipomea turpethum (Operculina turpethum)*	Turpeth, foe vao, St. Thomas lidpod
24	*Melissa officinalis*	Lemon balm
25	*Mentha piperita*	Peppermint
26	*Mentha royleana*	Royle's mint
27	*Piper nigrum*	Black pepper
28	*Plantago major*	Plantain
29	*Polygonum viviparum*	Alpine bistort
30	*Ptychotis ajowan (Trachyspermum ammi)*	Bishop's weed, ajowan, carom
31	*Quassia undulata*	Bitterwood
32	*Rheum emodi*	Himalayan rhubarb
33	*Saussurea lappa*	Snow lotus, kuth root, Arabian costus, costus
34	*Solanum nigrum*	Black nightshade
35	*Thymus linearis*	Himalayan thyme
36	*Verbascum thapsus*	Great mullein
37	*Zingiber officinale Roscoe*	Ginger

Table 2. More common antimicrobial herbs.

Activity	Herbs
Antibiotic	*Verbascum thapsus* (mullein), *Hypericum perforatum* (saint John's wort).
Antimicrobial	*Verbascum thapsus*, *Curcuma longa* (turmeric).
Antiseptic	*Hydrastis canadensis* (goldenseal), *Plantago* spp. (plantain).
Bactericidal	*Echinacea purpurea* (purple coneflower), *Melissa officinalis* (lemon balm), *Achillea millefolium* (yarrow).

5. Antimicrobial effects of phytochemicals

Up till now, an estimated 10,000 phytochemicals have been identified [52]. Though their number is increasing with the passage of time, many still remain unknown. Phytochemicals have been used by many researchers on selected microorganisms

[22,53,54] so as to study their antimicrobial effects. On the other hand, some researchers have experimented on a single class of phytochemicals; for example, phenolic acids (hydroxybenzoic and hydroxycinnamic acids) and their metal complexes have been studied for their antimicrobial effects [55,56]. Several other works describe their antibacterial, antiviral, antiprotozoal and antifungal characteristics [54,57–59]. Many researchers have documented that the affinity of phytochemicals towards the organic molecules in microbial cells may well be responsible for their antimicrobial activities. Trombetta et al. [60] worked on monoterpenes; they established that the lipid fraction of a microorganism's plasma membrane was disrupted, altering membrane permeability that resulted in leakage of intracellular materials. Although different mechanisms have been reported for phytochemicals as compared to the presently used antibiotics, yet a few investigators believe that the antimicrobial effects of phytochemicals and the exact mechanisms of action and specificity in antibacterial action of phytochemicals are not fully understood [61]. In another work, Wang et al. [62] attributed the microbial death to the damage induced to genomic DNA in addition to the alteration of cell membrane structure.

5.1. Phytochemicals' role in water disinfection

Malheiro et al. [63] studied the following seven phytochemicals as alternatives to disinfectants for planktonic and sessile cells of *Staphylococcus aureus* and *E. coli*: these are tyrosol, caffeic acid, ferulic acid, cinnamaldehyde, coumaric acid, cinnamic acid and eugenol. Cinnamaldehyde and eugenol produced remarkable results. Cinnamic acid was found very effective in control of sessile cells and was able to completely control the adhered bacteria with effects as good as peracetic acid and sodium hypochlorite, and more effective than hydrogen peroxide (all concentrations at 10 mM). Cinnamic acid was found to modify the surface properties of bacteria by making the surface less hydrophilic. It was also concluded that phytochemicals could additionally be used as dispersing agents of sessile cells. Anuj K. Saha [64] described a patented process for utilization of phytochemicals and sugar acids to create a media for usage in water purification filters. These filters release beneficial phytochemicals such as flavonoids, phytosterols, tannins, polysaccharides, saponins, polyacetylenes, and metallic nanoparticles, that are claimed to provide health benefits. Pervaiz [65] discussed disinfection of canal water using thymol, eugenol, and citric acid. Experiments were conducted both in the sunlight and without it. Citric acid at 100 ppm was the most efficient disinfectant in the presence of six hours of sunlight exposure and resulted in almost 75% disinfection of the heterotrophic bacteria. Consequently, citric acid may well be added to drinking water in household SODIS practices. Wali and Zafar [66] used thymol, and eugenol for disinfection of water from heterotrophic plate count microorganisms. Thymol was the most effective, with up to 2.6 log reduction at 300 ppm concentration of phytochemical and 60 min contact time. Higher than normal temperatures and higher pH favored greater log reduction. To perform experiments for disinfecting water with phytochemicals, Wali [47] devised a methodology to select suitable phyto-disinfectants from known antimicrobial phytochemicals. The general scheme is shown in Figure 2 below. Table 3 describes the relevant values and lists all these selected phytochemicals. A list of relevant antimicrobial phytochemicals with minimum inhibitory concentrations (MIC) of less than or up to 20 μg/mL was prepared and compared with conventional water disinfectants [68–88]. It is seen that the MIC values for the phytochemicals are slightly higher than those for conventional disinfectants. However, there is some uncertainty on the methods to measure the MIC values for the conventional water disinfectants [70]. As seen from Figure 3, allicin, berteroin, carvacrol, cinnamaldehyde, eugenol, sanguinarine, and thymol have significantly lower MIC values. When these phytochemicals are compared with those in Figure 4 it is easily discerned why various researchers have used these in their disinfection experiments. Also some of these phytochemicals are also present in the final list of phytochemicals prepared by Wali [47] as seen in Table 3.

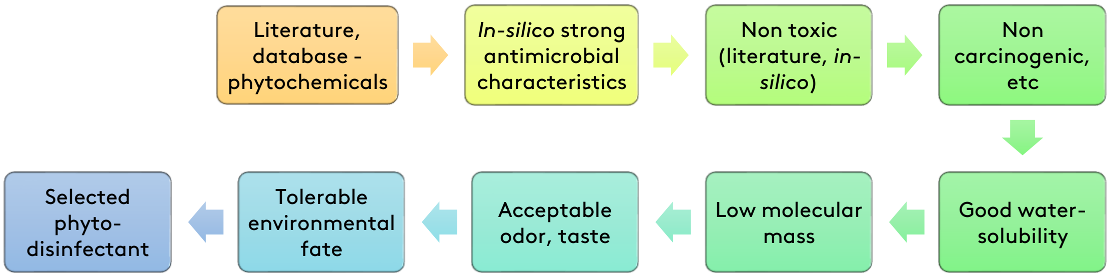

Fig.2. Methodology to search for suitable phyto-disinfectants.

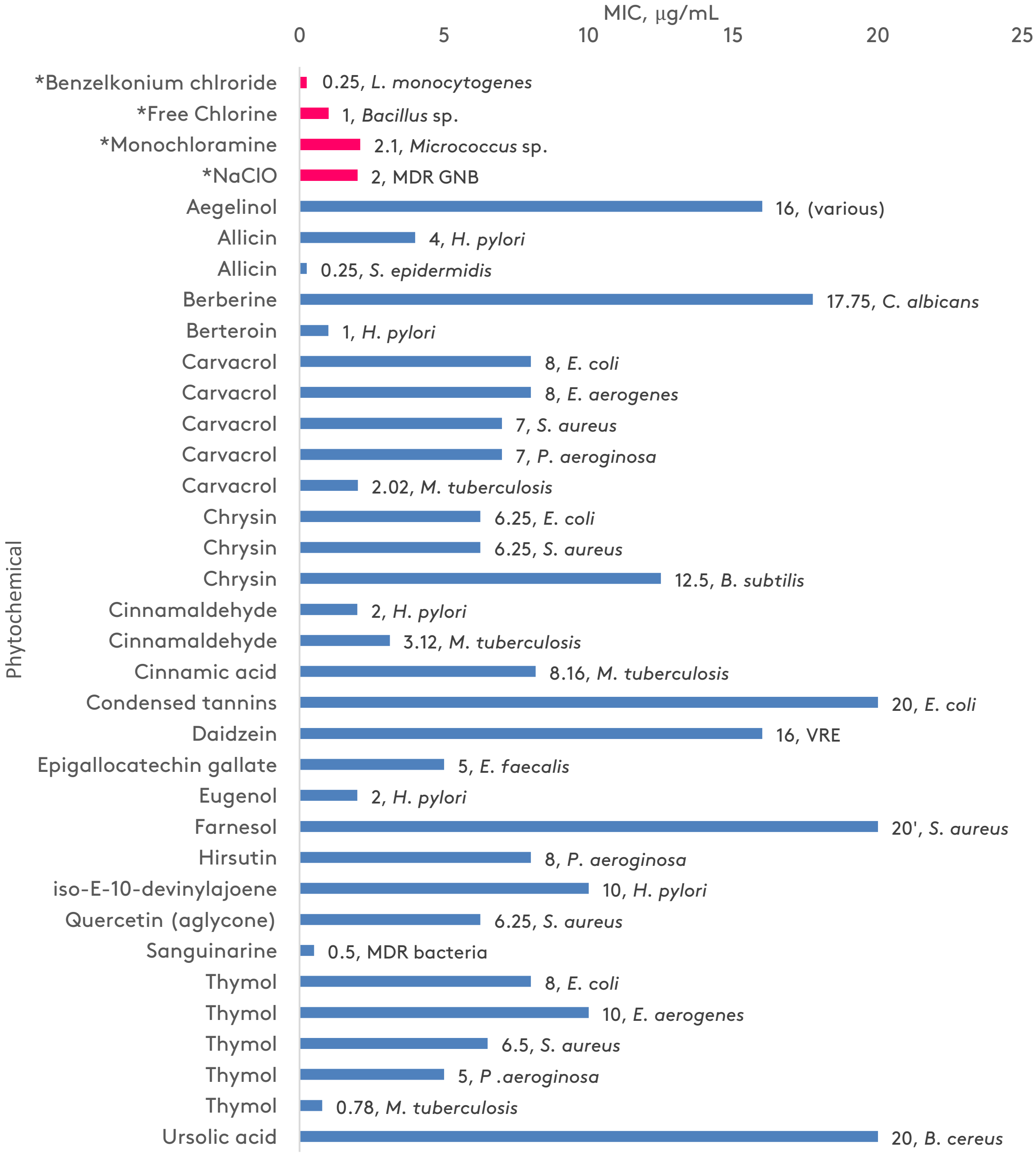

Fig. 3. *MIC values of particularly strong antimicrobial phytochemicals. The * sign is used for conventional water disinfectants. (Value marked ' is MBC).*

Table 3. List of selected phytochemicals suitable for water disinfection [67].

PHYTOCHEMICAL	***PubChem CID***	***Pa (Category)***	***Toxicity Rat oral (LD_{50}) mg/kg***	***Water Solubility mg/L***	***Odor/Taste***
1,8-Cineole (Eucalyptol)	2758	0.807 anti-infective	2480*	3500	Camphor/bitter-sweet
4-Isopropylphenol	7465	0.800 antiseptic	875 (mouse)	1102* (25°C)	
Alizarin		0.806 antiseptic	316 (wild bird)		
Aloin		0.717 antiviral	1566*		
Alpha-Terpineol	17100	0.785 antiseptic	5170	7100	Floral (lilac)/lime
Caffeic acid		0.782 antiseptic	LD_{Lo} intrapertl 1500	< 1000 (72°F)	
Carvacrol		0.898 antiseptic	810	1250* (25°C)	Thymol/smoke
Catechol (Pyrocatechol)	289	0.687 antiseptic	260	461,000 (25°C)	Phenolic/sweet-bitter
Citral	638011	0.756 antiviral	4960	1340 (37°C)	Lemon/bitter-sweet
Eugenol	3314	0.814 antiseptic	1930	2460	Cloves/pungent
Farnesyl acetone		0.760 antiviral	5780*	0.042* (25°C)	
Linalool	6549	0.711 antiviral	2790	1590 (25°C)	Floral/citrus
Menthol	1254	0.815 anti-infective	3180	456 (25°C)	Peppermint/peppermint
Menthyl salicylate	6970	0.938 antiseptic	2870*	0.143* (25°C)	
Myrcene		0.756 antiviral	>5000	4.09-5.60 (25°C)	Pleasant/citrus
Phytol		0.710 antiviral	6559*	0.003* (25°C)	
Thymol	6989	0.930 antiseptic	980	900 (20°C)	Thyme/aromatic
trans-Ferulic acid	445858	0.775 antiseptic	2754*	5970 (25°C)	

PHYTOCHEMICAL	***Stability at NTP***	***Carcinogenicity***	***Adsorption*** [@]	***Aquatic Fate***[#]	***MeSH***[$]
1,8-Cineole (Eucalyptol)	Good				Anti-infective agents, solvents
Alpha-Terpineol			No	13 d, 149 d	
Caffeic acid		Possibly Yes Group 2B	No	No	Antioxidants
Catechol (Pyrocatechol)	Discolors in air and light, aq. sol turns brown	Possibly Yes Group 2B	No	No	
Citral	Unstable to alkalis and strong acids		No	28 h, 12 d	
Eugenol	Darkens and thickens on exposure to air	Not classifiable	Yes	25 d, 183 d	Anti-infective agents, solvents
Linalool	Good		No	54 h, 20 d	Insecticides
Menthol			Yes	2 d, 18 d	Antipruritics
Myrcene			Yes	3.4 h, 4.6 d	
Thymol	Yes		Yes	13 d, 98 d	Anti-infective agents, local anti-infective agents, anti-fungal agents
trans-Ferulic acid			No	N/A	Cholagogues and choleretics, free radical scavengers, anticoagulants, antihypertensive agents, indicators and reagents, NSAI agents

Pa Probability as predicted by PASS for a given phytochemical to be active for a certain biological effect.

* Estimated values.

[@] To SS and sediments in water.

[#] Time required for volatilization from water surface – model river, model lake.

[$] Pharmacological Classification.

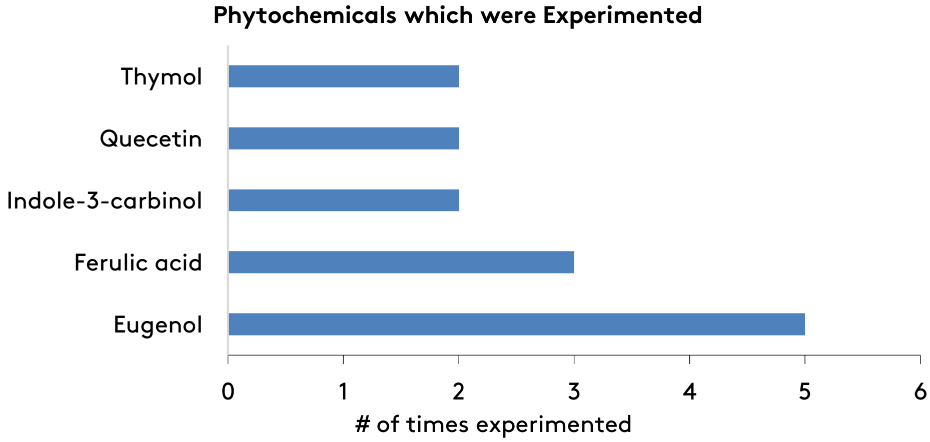

Fig. 4. The number of times as cited in this review, these phytochemicals and their metal complexes were tested on different planktonic and sessile microorganisms.

5.2. Prevention of Formation of Biofilms

Since the formation of biofilms is a significant characteristic of microorganisms and creates many problems, researchers have produced an extensive amount of literature to address this issue. Phytochemicals have also been utilized to study their biofilm prevention tendency and some of these have been found to be very effective. Ghaima et al. [89], studied the antibiofilm activity of water extract of flowers of *Calendula officinalis* against *Salmonella, Shigella dysenteriae, Shigella flexneri*, and *Shigella sonnei*. The extract decreased the adherent growth of bacteria on glass tubes, inhibited bacterial adhesion on polystyrene surface and caused biofilms detachment. Borges et al. [90], studied the activity of gallic and ferulic acids in the prevention of biofilms formed by four pathogenic bacteria (*E. coli, P. aeruginosa, S. aureus and L. monocytogenes*). Both the acids reduced the biofilm activity by greater than 70% for all the biofilms tested. Bacterial motility and adhesion were also reduced significantly. Both the acids caused permanent changes in membrane hydrophobic properties, decreased the negative surface charge and made possible the local rupture or pore formation in the bacterial cell membranes. The consequence was the leakage of essential intracellular constituents. Joana Monte et al. [22], used 7-hydroxycoumarin, indole-3-carbinol, salicylic acid and saponin against *E. coli* and *S. aureus* as planktonic cells and as biofilms. 7-hydroxycoumarin and indole-3-carbinol affected the motility and quorum-sensing activity of bacteria thereby interfering in biofilm formation. The combination of indole-3-carbinol with antibiotics produced synergistic effects against *S. aureus* resistant strains. Gomes et al. [91], investigated the utilization of natural based biocides (cumin aldehyde, eugenol, and indole-3-carbinol) on silicone and stainless-steel surfaces seeded with different strains of *S. aureus*. Eugenol was effective on stainless steel surface, while indole-3-carbinol at 10 × MIC and 5500 mg/L caused total CFU reduction of silicone and stainless-steel deposited bacteria. Although not as efficient as synthetic biocides, these phytochemicals are promising to be used as disinfectants for surfaces.

Li et al. [92] developed phytochemical based nanocomposites for the treatment of bacterial biofilms responsible for many infections. Cross-linked polymeric scaffolds were used as a delivery strategy. It significantly improved the antimicrobial efficacy of phytochemicals against both planktonic bacteria and biofilms. It was found that phytochemicals with lower log P value were more effective for that delivery system. Lower log P value and absence of phenolic hydroxyl groups provided particularly low cytotoxicity nanocomposites. Phytochemical nanocomposites, for example, linalool and methyl eugenol were especially noticeable to address wound biofilm infections.

5.3. Cost-wise comparison

Table 1S (Supplementary Material, Online Resource) lists those disinfectant phytochemicals which have some antibacterial, bacteriostatic or bactericidal effects especially against *E. coli* and generally against *Enterobacteriaceae* and *Enterococci* [53,54,57,58,93]. The interest here lies in using these phytochemicals to kill the water-borne microorganisms. The phytochemicals mentioned in Table 1S are either obtained from synthetic or from natural sources. It is noted that some of the antimicrobial phytochemicals present for example in ginger, St. John's wort, noni, hops, tamarind, and propolis are very expensive. On the other hand, several others are quite affordable, for example, citric acid, coumarin, eugenol, menthol, thymol etc. A survey of pertinent research works revealed that expensive phytochemicals have seldom been used in disinfection experiments. Thymol, for example, has been calculated as almost 450 times more costly than the same amount of Cl_2 gas (a conventional disinfectant). Affordability needs further consideration. However, expensive phytochemicals should also be used in disinfection experiments to elucidate their true potential. In other words, cost-effectiveness must not be considered as a hurdle in search for effective disinfectant phytochemicals.

5.3. Toxicity of phytochemicals

As the phytochemical will be consumed along with drinking water the emphasis here is therefore on the oral route of exposure. A compilation of toxicity data of various phyto-disinfectants is presented in Table 2S (Supplementary Material, Online Resource). Alpha-terpineol, citral and myrcene have LD_{50} values at around 5000 mg/kg of body weight; it is also seen that linalool and menthol have values at about 3000 mg/kg of body weight. These values are fairly higher than the common conventional disinfectants and this is advantageous for these phytochemicals when applied as disinfectants to make water potable. Eugenol has 1930 mg/kg, and caffeic acid has 1500 mg/kg (intraperitoneal value). Whereas 4-isopropylphenol, carvacrol, and thymol have values slightly less than 1000 mg/kg of body weight. However, it is also clear from the data that 1,4-naphthoquinone, alizarin, catechol, and trans-ferulic acid all have LD_{50} values less than 320 mg/kg of body weight. These values are for animals, mostly rats and mice. In comparison to these values, the oral LD_{50} value for calcium hypochlorite (a common water disinfectant) is established at 850 mg/kg of body weight for rats [94]. Many a phytochemical which may possess some potential for use in water disinfection has been classified as very toxic to humans (A reference mass for an average human being is taken here as a 70 kg (150 lb) person). Thus carvacrol, 1,8-cineole, and menthol are very toxic (4.4 for each; the probable oral lethal dose is 50 – 500 mg/kg, i.e., between one teaspoonful and one ounce) [95]. Catechol is also very toxic (4.0) [96]. Terpineols are moderately toxic (3.3; the probable oral lethal dose is 0.5 – 5 g/kg, i.e., between 1 ounce and 1 pint (or 1 lb.)) [95]. Some antimicrobial phytochemicals are consumed along with food. Alpha terpineol had no adverse effects at a dose of 500 mg/kg of body weight per day [97]. Individual consumption of linalool is 0.01822 mg/kg of body weight/day [98]. The therapeutic dose of citral at 500 μg/kg is regarded as acceptable daily intake. Hence alpha terpineol, linalool, and citral are not regarded toxic. Caffeic acid and catechol are evaluated as "possibly carcinogenic to humans" and are classified in Group 2B [99]. The lethal doses of a few antimicrobial phytochemicals are known for humans. For both catechol and menthol, the probable oral lethal dose is 50 to 500 mg/kg, for a 70 kg person [100]. Thymol is thought to lie near between toxicity classes 3 & 4 /moderately & very toxic/ [101]. Eugenol is not corrosive like phenol but ingestion would cause gastroenteritis. Systemic toxicity of eugenol is similar to but less than that of phenol (perhaps because of its insolubility in water) [101]. Unfortunately, at present, sufficient pertinent data for the toxicity of antimicrobial phytochemicals to humans is not available. It might be due to the colossal number of phytochemicals being discovered. Nevertheless, these values can help determine the right quantity to be utilized in the disinfection of water.

5.4. Isolation of Phytochemicals from Herbs and Extracts

Because the aims and objectives are to study the effects from a pure single phytochemical, therefore only isolation techniques will be discussed; the extraction techniques will not be analyzed here [102]. Although purified analytical grade phytochemicals are available through different manufacturers, they are generally very expensive. If a simple route of synthesis of a desired phytochemical is available, it ought to be preferred by the researchers. However, in the absence of such preparatory methods, isolation of phytochemicals from their herbs may be desired by the researchers. Isolation and purification steps are generally laborious and time consuming. As an estimate, for each of the ten phytochemicals in some herb, generally two or three have antimicrobial effects [103]. For isolation of phytochemicals, researchers prefer dry herbs and seeds. Fresh herbs are not used as they contain much water and the extracts obtained are diluted. Essential oils (EO) are generally obtained through distillation of herbs and are quite useful as they contain many phytochemicals; however, the isolation of each phytochemical will be arduous, for example refer to [104]. If the aim is to study the effect of a single phytochemical, the EO therefore, should not be used. High Performance (or Pressure) Liquid Chromatography (HPLC) is used for liquid extracts. Analytical HPLC is unsuitable for isolation of phytochemicals due to extended time, cost and lower yield. A preparatory scale HPLC at the minimum is required for separation. The phytochemical that is to be separated using the HPLC, is already known to the experimenter. However, for identification purposes the 'standard' of that phytochemical (i.e., highly purified phytochemical, which is costly) is also utilized. HPLC spectrograph displays peaks and when it is like that of the standard, the said phytochemical is identified. Voluminous quantities of herbs are required to isolate the phytochemicals which make up a tiny percentage of the whole plant material. After the analytical HPLC peaks are obtained, they are studied for guidance of the time when they appear on spectrograph. Then, the whole extract is passed through a column called "open column chromatography apparatus". During isolation of the phytochemical by using this apparatus, the time of the product elution from the bottom of the column will correspond to the time on the analytical HPLC spectrograph. Open column chromatography is a feasible option for isolation purposes [105]. It is normally a few inches wide and about a meter or two tall; the glass column is filled with packing material made of silica or alumina, and the extract is poured from top. After discarding the first few mL, one by one the separated fractions of extract are taken out from the bottom tap. For example, separately taking 5 mL then again 5 mL or taking samples each after a fixed period of time. These extracts contain different phytochemicals and hence a fair extent of separation is achieved. It may be mentioned that this process is costly when adopted on a commercial scale. If a sample is volatile then Gas Chromatography Mass Spectrometry (GCMS) is used. This is of course, an expensive technique [106]. On the other hand, the Liquid Chromatography Mass Spectrometry (LCMS) is regarded as the first-rate method for isolation and identification of compounds [107]. Lastly the instrument, Fourier Transform Infra-Red Spectrophotometer (FTIR), can be used for characterization of the isolated phytochemical. Unlike the other instruments discussed here, it cannot isolate a phytochemical from its extract, but it identifies the functional groups and molecular fragments in an organic compound, which may help in its identification. Researchers have used quite a number of modern isolation technologies, and some have been commercialized as well. Adeeyo et al. [6] discussed some modern techniques and Zhang et al. [108] reviewed various isolation techniques and discussed those on the basis of mechanism of separation. These are outlined in the following Table 4.

6. *Phytochemical-metal complexes for water disinfection*

Another important advancement in disinfection of water by phytochemicals is the utilization of their metal complexes. It may be noted that many phytochemicals are not appreciably soluble in water [112], and consequently, have lesser antibacterial properties as compared to their more soluble metal salts. Due to the formation of poles within a metal complex molecule, it generally achieves appreciable solubility in polar solvents which aids in the disinfection process. A few examples highlighting the increased solubility have been presented in the following Table 5.

Table 4. Modern isolation techniques of phytochemicals from their extracts [6, 108].

Mechanism	Application Technology	Advantages and Disadvantages
Difference in adsorption affinities of phytochemicals towards adsorbent.	Column Chromatography (CC) Thin Layer Chromatography (TLC) High Performance Thin Layer Chromatography Preparative Gas Chromatography (Prep-GC)	Silica gel: versatile for many phytochemicals. Some cases of irreversible adsorption and severe tailing. Alumina: better for polar phytochemicals. May catalyze dehydration, decomposition, or isomerization. Macroporous resins: higher adsorption capacity, lower cost, easier regeneration. $AgNO_3$ impregnation: phytochemicals with more π electrons. Rapid analysis, easy sample preparation. Compounds with low polarities, to detect fake products [109]. High efficiency and fast separation. Suitable for volatile phytochemicals. Commercial Prep-GC not available. Consumes large volume of carrier gas, decomposition of thermolabile phytochemicals at high temperature, fraction collection difficult and low.
Partition coefficient: relative solubility in two different immiscible phases	Partition Chromatography. Includes Centrifugal Partition Chromatography (CPC), and Counter-Current Chromatography (CCC) stationery liquid phase is held by gravity or centrifugal force. It is further divided into High-Speed CCC (HSCCC) and High Performance CCC (HPCCC).	CCC eliminates irreversible adsorption and peak tailing, has high loading capacity and sample recovery, minimum risk of sample denaturizing, and low solvent utilization. CCC has a relatively narrow polarity window.
Different molecular size	Membrane Filtration (MF), Gel Filtration Chromatography (GFC) also called Gel Permeation Chromatography (GPC) or Size Exclusion Chromatography.	GFC separates wide variety of phytochemicals in both aqueous and non-aqueous solvents.
Ionic strength: separate molecules due to differences in net surface charge.	Ion Exchange Chromatography (IEC)	Cation exchange resins are good for alkaloids, while anion exchange resins for organic acids, and phenols
Distillation under vacuum at temperatures much below normal boiling point.	Molecular distillation	Use for thermosensitive and high molecular weight compounds.
Supercritical fluid as mobile phase in column. **SC fluids have high solubilization and diffusivity, and low viscosity, which causes rapid and efficient separation.**	Supercritical Fluid Chromatography (SFC)	Integrates benefits of GC and LC. For non-volatile and thermally labile compounds to which GC and LC may not be appropriate.
Making complementary cavities with memory of size, shape and functional groups of the template molecules when they are removed from molecular imprinted polymer.	Molecular Imprinted Technology (MIT)	High selectivity, low cost, easy preparation.
Multiple columns with static beds. Rotary valves periodically switch the inlet and outlet.	Simulated Moving Bed (SMB) Chromatography	Continuous operation. Suitable for large-scale with lower solvent consumption over a shorter time
Combination of multiple columns with different stationary phases	Multi-dimensional chromatographic separation	Very high separation efficiency. Commercially available.
Chromatography profiles obtained from chemical components in extracts [110].	Chromatographic Fingerprinting	Similar chemical components with different identifiable chemical characteristics.
DNA analysis [111].	DNA Fingerprinting	Identify fake products. Availability of intact genomic DNA from herbs

Table 5. Increased solubility of the phytochemical-metal complexes than phytochemicals.

Phytochemical-Metal Complex	Molecular Mass of Complex (g/gmol) [a]	Solubility of Phytochemical (g/L) [113]	Solubility of Complex at pH 7.0 (g/L) [b]
Eugenol silver	272.1	Eugenol, 2.46 (25°C, exp)	2.48
Sodium ferulate	216.2	Ferulic acid, 5.97 (25°C, est)	216.2
Silver lactate	197.9	Lactic acid, 1000 (exp)	59,766
Copper (II) quercetin	668.0	Quercetin, 0.06 (16°C, exp)	2,786
Zinc salicylate	341.6	Salicylic acid, 2.24 (25°C, exp)	341.6
Calcium tartrate	190.2	Tartaric acid, 582 (20°C, exp)	50,023
Thymol sodium	173.2	Thymol, 0.9 (20°C, exp)	0.35

'est': estimated, 'exp': experimental.

[a] Obtained from MarvinSketch 15.5.4 (ChemAxon)

[b] Estimated from the solubility plugin in MarvinSketch 15.5.4 (ChemAxon)

6.1. Antimicrobial Phytochemical-Metal Complexes

Effects reported on the antimicrobial activities of organic compounds in the presence of metal ions can either be positive or negative [114]. So, phytochemical-metal complexes must be checked experimentally for their disinfection efficacy. Although, many antimicrobial phytochemical-metal complexes are reported in literature, however, they have seldom been used as disinfectants for drinking water. Weinberg [114], lists a few antibacterial phytochemicals metal complexes that are effective against certain microorganisms. For example, juglone (Cu^{++} complex) is active against *B. subtilis*. Metal complexes of quinine, and citrate (Mg^{++} and Mn^{++} complexes) are not effective against the tested microorganisms. Some of the phytochemicals were not tested at that time, e.g., embellin, lapachol, plumbagin, and anacardic acid. Similarly, Weinberg [114], also lists a few phytochemicals with anti-viral effects that can form metal complexes, for example, juglone, morin, and lapachol (lapachol had not been tested experimentally). The antimicrobial characteristics of flavonoid metal complexes are widely reported. Many of the flavonol-metal ion complexes have been found to exhibit antimicrobial activity. [13,115,116]. However, the exact mechanism for such activity is yet to be fully clarified. The presence of metal ions in the complexes favors their binding to the enzymes in a covalent manner, thereby exhibiting better inhibitory activity than the parent flavonoid [115]. Another important mode proposed for the inhibition of bacterial growth by flavonoid–metal ion complexes is their nonspecific intercalation with the DNA double helix. This in turn alters the gene expression leading to seizure of cell division [117]. Bravo and Anacona [116] examined the antibacterial activities of Mn, Co, Cd and Hg complexes of quercetin. Cadmium-quercetin complex had good inhibition of bacterial growth, but the mercury-quercetin complex was found to exhibit powerful growth inhibitory effect against the four microorganisms (*E coli, S aureus, B cereus, K pneumoniae*) tested. A study on the antimicrobial activity of quercetin against *S. aureus*, methicillin resistant *S. aureus*, and *S. epidermidis* has indicated that quercetin shows superior and selective antibacterial activity against the above-mentioned microorganisms at a concentration of 50 μM [118]. Mn, Hg, Co, and Cd complexes of quercetin have been found to show a better bactericidal effect against all the microorganisms tested [119]. Not all flavonoid-metal ion complexes have been reported to possess superior antibacterial effects as compared to their parent flavonoids. For example, lanthanide, gadolinium, and lutetium complexes of morin have been reported to possess antibacterial activity. However, the lanthanide and gadolinium complexes have demonstrated a lesser antibacterial activity than morin unlike quercetin-metal ion complexes [115]. In many cases the metal complexes of flavonoids result in better pharmacological activities and the complexes possess better stability both *in-vitro* and *in-vivo* [119]. Khater et al. [120] reviewed the biological properties of flavonoid metal complexes. Srivastava et al. [121] reported the antimicrobial nature of Cd quercetin complex. Wali and Zafar [66] checked disinfection efficacy of calcium ferulate on heterotrophic bacteria in water. Up to

85.2% reduction for 300 ppm of calcium ferulate after 60 min of contact time was reported.

6.2. Cost-wise comparison

It is well known that the prices of salts are generally less than those of phytochemicals. For example, cadmium chloride hydrate had a relative price of 0.5 (98% purity) and for lanthanum chloride.7H_2O was 0.2 (+99% purity). Many metallic salts are highly toxic as well. Hence an important consideration for disinfection purposes will be non-toxicity of the phytochemical metal complex. Not all phytochemicals form complexes with metal ions. For a number of phytochemicals, whose metal complexes have been reported by different researchers, a list has been prepared in Table 3S (Supplementary Material, Online Resource) wherein the *relative prices* of these phytochemicals are presented. Some other important phytochemicals whose metal complexes have been reported in the literature were studied. However, due to the lengthy and time-consuming process of their synthesis and thereby pushing the cost upward during their preparation, those metal complexes are not included in Table 3S. They are as follows: 5-amino-8-hydroxy-1,4-naphthoquinone, N-amino-quinolone derivatives, tridentate Schiff bases, ONO donor ligands containing indole and coumarin moieties, formyl chromone Schiff bases, Pd chromone Schiff bases, and thiazole and quinoline moieties. These price factors are calculated on the same basis as in the aforementioned Table 1S (Supplementary Material). As shown earlier in Table 1S, the prices are significantly higher than those of conventional disinfectants. However, due to easier synthesis of metal complexes, many phytochemicals can be utilized to form complexes at fairly affordable prices.

6.3. Toxicity of phytochemical-metal complexes

Pertinent toxicity data on phytochemical-metal complexes is usually unavailable; as an alternative, toxicity of the phytochemicals and salts may be checked separately. Salts of citric acid are fairly tolerable. For example, a dose containing 0.64g sodium citrate per 100mL is available in injectable form [122]. Some acids are toxic, for example lactic acid [123], whereas others may irritate the skin, like malic acid [123]. In the case of flavonoids, their varied effects on humans have been studied. The more significant ones include quercetin, kaempferol, myricetin, apigenin, and luteolin, which have been studied for their effects in reducing the risk of cancer. However, no measurable effects were observed [124]. Quercetin is not classifiable as to its carcinogenicity to humans [125]. Kaempferol is expected to have potentially beneficial effects in preventing estrogen imbalance diseases [126].

7.Conclusions

Many different species of plants showed disinfection characteristics. Species that contained antimicrobial phytochemicals, for instance, *Ocimum sanctum*, *Trachyspermum ammi*, *Zingiber officinale*, and *Azadirachta indica* remarkably reduced the microbial load at rather higher concentrations. *M. oleifera*, though unsuitable as a disinfectant, is considered effective in terms of removal of turbidity. Combination of plant extracts with SODIS produced good results. Eugenol is of interest to many researchers for the disinfection of water. Others have used ferulic acid, thymol, quercetin and indole-3-carbinol for the same purpose. However, as discussed already, these were not necessarily the most effective ones. Several researchers have worked on the utilization of phytochemicals for the prevention of biofilms on surfaces. While this aspect is very pertinent in the context of water treatment, more work on use of phytochemicals as disinfectants for planktonic cells is required to understand further their disinfection capabilities for drinking water. It is believed that metal complexes of phytochemicals can serve as more effective water disinfectants as compared to plant extracts. It is proposed that further work on antimicrobial metal complexes should be carried out to determine their efficiency as disinfectants for drinking water. As discussed earlier, the flavonoid-metal complexes could potentially be developed into efficient water disinfectants. It is evident from Tables 1S and 3S, that many phytochemicals are quite expensive. Higher cost might become a barrier to use phytochemicals for disinfection purposes. The toxicity values for conventional disinfectants are low; inversely, the corresponding values for phytochemicals are fairly high. However, as seen from the results of various researchers, high doses of phytochemicals have been used to achieve the desired disinfection effects. Therefore, there is a need to find more antimicrobial phytochemicals which are nontoxic. To summarize, it is deduced from the results of various works that currently phytochemicals along with soluble metal salts or with SODIS, might offer a feasible disinfection option. Another important theme for the successful use of phytochemicals as disinfectants of water may possibly be the designing and building of an efficient contactor vessel. In that case, water would be passed through a fixed bed of disinfectant medium. Such technologies could be used for example in far-flung areas where people are hesitant to use conventional disinfectants.

8. Acknowledgements

The author is very grateful to Mr. Wali Muhammad for correcting the English of this manuscript. The author is greatly obliged to Prof. Dr. Muhammad Mahmood Ahmed, Prof. Dr. Muhammad Ali Maud, Prof. Dr. Rubina Gillani, Dr. Humayun Ejaz and Dr. Abdul Rauf, all at the University of Engineering and Technology, Lahore. The author is also grateful to Prof. Dr. Zeb Siddique (Lahore College for Women University).

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