Selection Process of Phytochemicals for Utilization in Disinfection of Drinking Water

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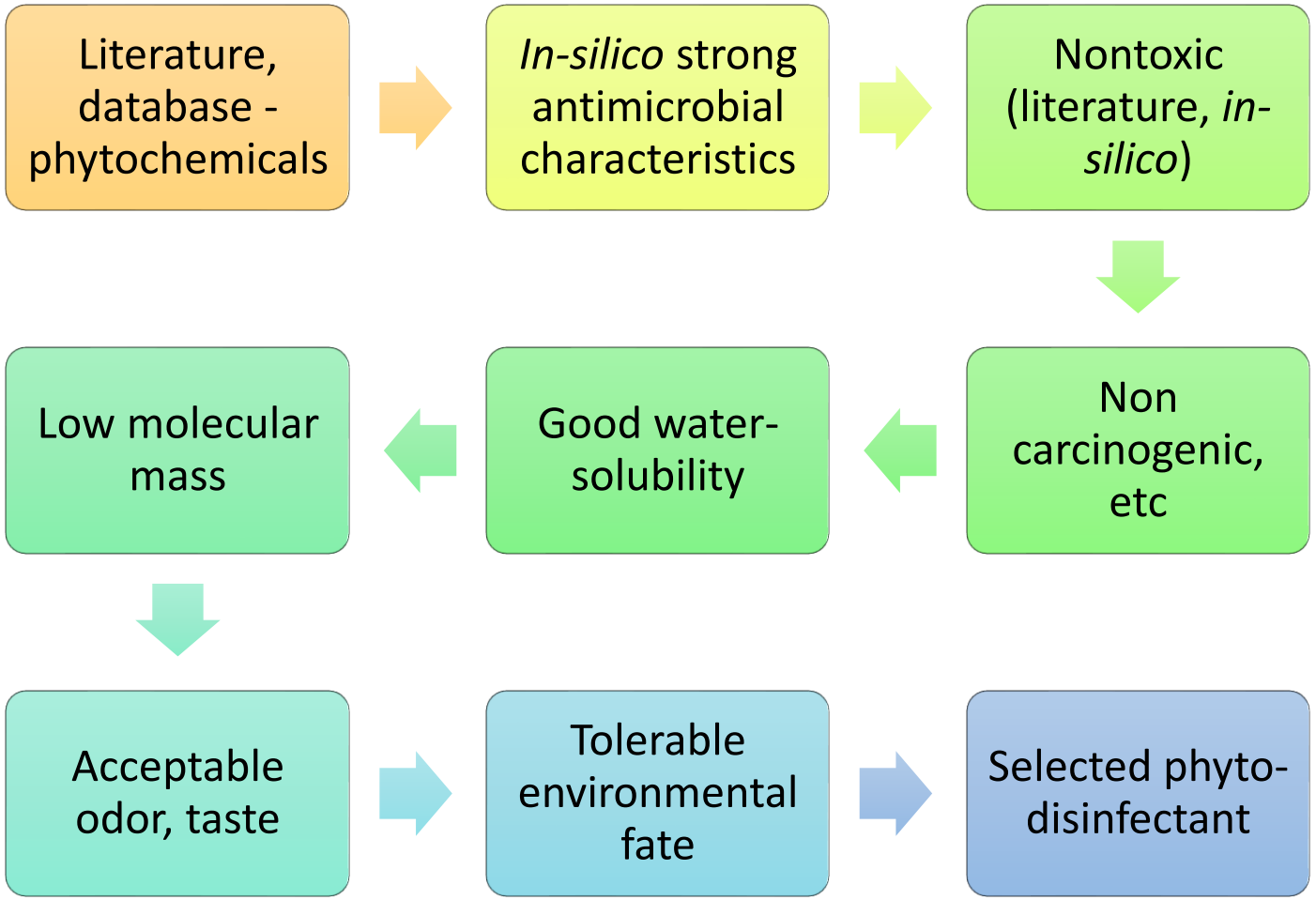
Research Work Objectives

- To identify phytochemicals which possess strong antimicrobial effects and can form metal complexes.
- To investigate the efficacy of phytochemicals and their metal complexes as disinfectants for drinking water under various conditions.

Development of Selection Criteria

- Following resources were utilized for the purpose of selecting suitable phytochemical and phytochemical-metal complexes that possess antimicrobial effects:
  - Literature Search [1-5]
  - Database [6-13]
  - Software [6-13]
- These phytochemicals were checked for cost, availability, water solubility, stability, lipophilicity, etc.
- The molecular structures of these phytochemicals were drawn (ChemAxon) and they were checked *in-silico* for antimicrobial activity and toxicity using web-resources (PASS and GUSAR).

Development of Selection Criteria



Selected Phytochemicals

PHYTOCHEMICAL	MICROBES TESTED HERBS	Description "SMILES"	PASS PREDICTIONS Pa.....Pi.....EFFECTS	PASS PREDICTIONS Pa.....Pi.....EFFECTS
1,4-Naphthoquinone	S. aureus, S. intermedius, S. epidermidis, B. cereus, S. uberis, E. faecelis were affected	Antibacterial drug, fungicide "c1ccc2c(c1)C(=O)C=CC2=O"	0.610...0.017...Antiviral (Picornavirus) 0.549...0.010...Antihelmintic (Nematodes) 0.479...0.008...Antiviral (adenovirus)	0.910...0.005...Shivering 0.890...0.004...Urine discoloration 0.881...0.008...Twitching
1,8-Cineole	EO (C) of cardamom and eucalyptus. Bay and Myrtus communis	Antitussive "CC1(C2CCC(O1)(CC2)C)C"	0.807...0.005...Anti-infective 0.723...0.004...Antiprotozoal 0.693...0.006...Antiparasitic 0.675...0.004...Antihelmintic 0.663...0.006...Antiseptic 0.462...0.030...Antiprotozoal (Leishmania)	0.875...0.012...Drowsiness 0.867...0.005...Non-mutagenic. Salmonella 0.845...0.017...Sleep disturbance
1-Menthyl salicylate	Wintergreen (contains methyl salicylate)	"CC1CCC(C(C1)OC(=O)c2ccccc2O)C(C)C"	0.938...0.003...Antiseptic 0.839...0.004...Anti-infective 0.681...0.007...Antiviral (Influenza) 0.554...0.010...Antihelmintic (Nematodes) 0.557...0.023...Antifungal 0.517...0.014...Antiparasitic 0.488...0.017...Antibacterial 0.441...0.012...Antihelmintic 0.437...0.022...Antiviral (Herpes)	0.976...0.001...Ulcer. aphthous 0.969...0.002...Irritation 0.959...0.004...Muscle weakness

Selected Phytochemicals

Ascorbic acid	Many fruits, vegetables	Antioxidant, vitamin "C([C@@H]([C@@H]1C(=C(C(=O)O1)O)O)O)O"	0.567...0.010...Antiviral (Rhinovirus) 0.466...0.023...Antihelmintic (Nematodes)	0.981...0.002...Acidosis. metabolic 0.976...0.004...Toxic. vascular 0.969...0.003...Acidosis
Carvacrol	28 EO tested against E. coli O157:H7, L. monocytogenes, S. aureus, S. Typhimurium. E. coli, S. Typhimurium. Ptychotis ajowan, EO Thymus vulgaris L.	Antifungal agent, antiseptic drug, nematicide "Cc1ccc(cc1O)C(C)C"	0.898...0.003...Antiseptic 0.784...0.005...Anti-infective 0.722...0.004...Antihelmintic (Nematodes) 0.525...0.013...Antiparasitic 0.523...0.020...Antiviral (Influenza) 0.497...0.006...Antihelmintic	0.969...0.007...Toxic. respiration 0.945...0.002...Hematemesis 0.924...0.003...Ulcer. aphthous
Catechol	Argania spinosa (argan)	Antibacterial drug, antiseptic drug "c1ccc(c(c1)O)O"	0.687...0.005...Antiseptic 0.618...0.015...Antiviral (Picornavirus) 0.589...0.007...Antihelmintic (Nematodes) 0.582...0.014...Anti-infective 0.547...0.002...Antihelmintic (Fasciola) 0.535...0.008...Antiprotozoal (Amoeba)	0.926...0.004...Shivering 0.924...0.003...Urine discoloration 0.920...0.003...Ulcer. aphthous
Cinnamaldehyde	28 EO tested against E. coli O157:H7, L. monocytogenes, S. aureus, S. Typhimurium	"c1ccc(cc1)/C=C/C=O"	0.520...0.016...Antiprotozoal (Trypanosoma)	0.923...0.003...Irritation 0.841...0.022...Shivering 0.803...0.040...Twitching

Selected Phytochemicals

Citric acid	Total count, yeast and mold, E. coli O157:H7, L. monocytogenes	Antibacterial drug, anticoagulant "C(C(=O)O)C(CC(=O)O)(C(=O)O)O"	0.592...0.007...Antiviral (Rhinovirus) 0.575...0.025...Antiviral (Picornavirus)	0.983...0.004...Toxic. respiration 0.965...0.003...Acidosis. metabolic 0.906...0.009...Acidosis
Coumarin	Nine pathogenic fungal and eight pathogenic bacterial with synthetic coumarin derivatives	Anticoagulant "c1ccc2c(c1)ccc(=O)o2"	0.628...0.005...Antihelmintic (Nematodes) 0.585...0.008...Antiviral (Rhinovirus) 0.571...0.005...Antiprotozoal (Amoeba) 0.531...0.011...Antiseptic 0.532...0.015...Antiprotozoal (Trypanosoma) 0.503...0.015...Antiparasitic 0.504...0.024...Antiprotozoal (Leishmania)	0.831...0.025...Shivering 0.813...0.012...Hematemesis 0.782...0.011...Occult bleeding
Eugenol	E. coli, S. Typhimurium. E. coli O157:H7, L. monocytogenes. EO of Eugenia caryophylata, Betel pepper, oil of clove	Analgesic "COc1cc(ccc1O)CC=C"	0.814...0.004...Antiseptic 0.562...0.005...Antiprotozoal (Amoeba) 0.561...0.017...Antiprotozoal (Leishmania) 0.473...0.035...Antifungal	0.936...0.005...Euphoria 0.857...0.003...Fatty liver 0.857...0.005...Apnea

Selected Phytochemicals

Thymol	E. coli, S. Typhimurium. E. coli O157:H7, S. aureus, S. Typhimurium, L. monocytogenes. Thymus vulgaris L. EO, Ptychotis ajowan	Antifungal agent, antiseptic drug, nematicide "Cc1ccc(c(c1)O)C(C)C"	0.930...0.003...Antiseptic 0.829...0.005...Anti-infective 0.765...0.003...Antihelmintic (Nematodes) 0.588...0.009...Antiparasitic 0.551...0.005...Antihelmintic	0.968...0.008...Toxic. respiration 0.929...0.003...Hematemesis 0.924...0.003...Ulcer. aphthous
trans-Anethole	Foeniculum vulgare Mill + Gaertn	"C/C=C/c1ccc(cc1)OC"	0.571...0.008...Antihelmintic (Nematodes) 0.563...0.016...Antiviral (Influenza) 0.499...0.019...Antiprotozoal (Trypanosoma) 0.483...0.027...Antiprotozoal (Leishmania)	0.866...0.014...Shivering 0.803...0.009...Extrapyramidal effect 0.765...0.008...Hypercholesterolemic
trans-Caffeic acid		Antioxidant "c1cc(c(cc1/C=C/C(=O)O)O)O"	0.782...0.004...Antiseptic 0.678...0.004...Antihelmintic (Nematodes) 0.548...0.017...Antiviral (Influenza) 0.520...0.010...Antituberculosic 0.515...0.022...Antiprotozoal (Leishmania)	0.902...0.004...Urine discoloration 0.885...0.005...Hematemesis 0.877...0.005...Ulcer. aphthous
trans-Ferulic acid	C. albicans.	Antihypertensive agent "COc1cc(ccc1O)/C=C/C(=O)O"	0.775...0.004...Antiseptic 0.583...0.008...Antihelmintic (Nematodes) 0.554...0.018...Antiprotozoal (Leishmania) 0.501...0.012...Antituberculosic 0.501...0.022...Antiviral (Influenza) 0.496...0.019...Antimycobacterial	0.839...0.007...Urine discoloration 0.819...0.004...Irritation 0.812...0.004...Hypercholesterolemic

Selected Phytochemicals

Phytochemical	*Rat IP LD_{50} (mg/kg)*	*Rat IV LD_{50} (mg/kg)*	*Rat Oral LD_{50} (mg/kg)*	*Rat SC LD_{50} (mg/kg)*
1,8-Cineole	389.10	59.30	2480.0	314.90
4-Isopropylphenol	289.3	63.74	927.3	496.3
Alizarin	1126	116.7	1079	1095
Aloin	1087.0	999.90	1566.0	1372.0
Farnesyl acetone	299.40	257.20	5780.0	3178.0
Menthyl salicylate	927.60	44.120	2870.0	196.80
Phytol	2133.0	198.30	6559.0	2194.0
trans-Caffeic acid	890.2	361.4	2386.0	574.7
trans-Ferulic acid	682.30	224.40	2754.0	1058.0

Selected Phytochemicals

PHYTOCHEMICAL	Pa (Category)	Toxicity Rat oral (LD50)	Water Solubility	Odor / Taste	Stability at NTP	Carcinogenicity	Adsorption^	Aquatic Fate*	MeSH Pharmacological Classification
Thymol	0.930 antiseptic	980mg/kg	900mg/L	Thyme / aromatic	Yes	---	Yes	13, 98	Anti-infective, antifungal
Eugenol	0.814 antiseptic	1930mg/kg	2460mg/L	Cloves / pungent	Darkens & thickens in air	Not classifiable	Yes	25, 183	Anti-infective, solvent
trans-Ferulic acid	0.775 antiseptic	2754mg/kg (GUSAR)	5970mg/L (25°C)	---	---	---	No	N/A	Cholagogue, choleretic, free radical scavenger, anticoagulant, antihypertensive

^ to SS and sediments in water
* Volatilization from Water Surface – (model river, model lake) days
--- No data reported in major databases.

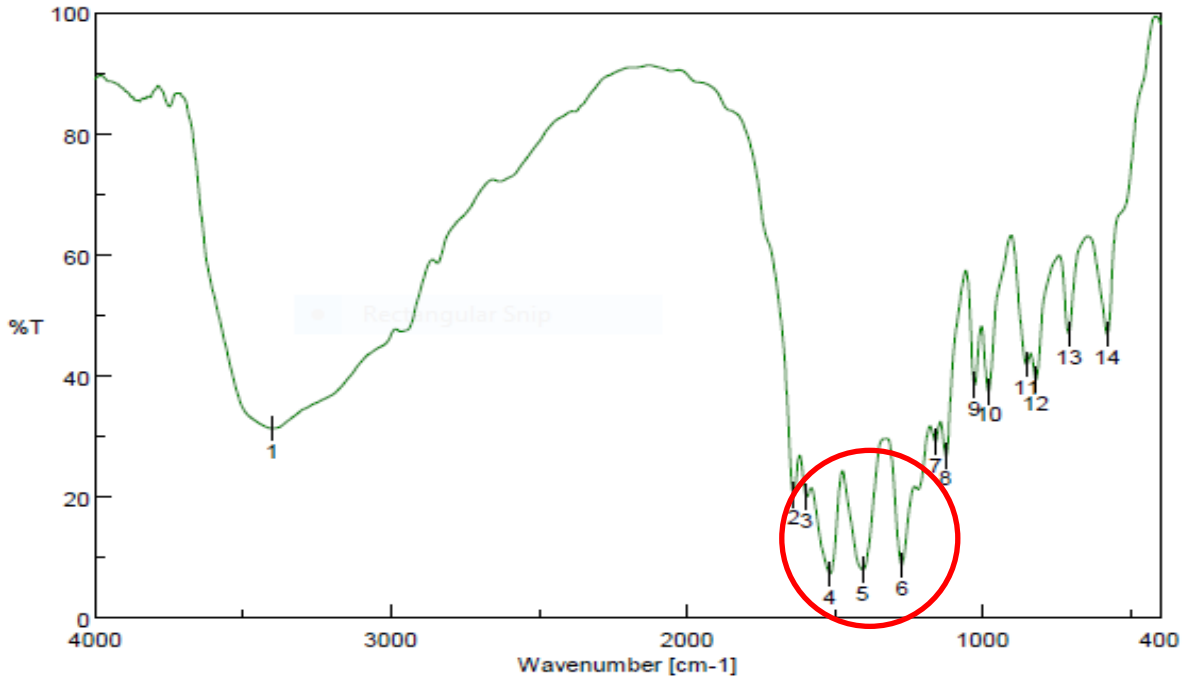
Selected Phytochemical-Metal Complexes

- Being more soluble in water, phytochemical-metal complexes might be better disinfectants than phytochemicals.
- The molecular structures of selected metal cations Ag^{+}, $Ca^{2+}$and Zn^{2+} complexed with selected phytochemicals were either identified from literature or drawn in software and checked for errors.
- Antimicrobial activity and toxicity values were retrieved from web resources (PASS and GUSAR) by providing the input of molecular structures of the phytochemical-metal complexes.
- As a result of the above-mentioned work, following list of phytochemical-metal complexes was prepared:

Selected Phytochemical-Metal Complexes

S #	PHYTOCHEMICAL-METAL COMPLEX	DESIRABLE PASS PREDICTIONS Pa	Pi	EFFECTS	UNDESIRED PASS PREDICTIONS Pa	Pi	EFFECTS
1	Calcium ferulate	0.959	0.003	Antiseptic	0.861	0.010	Neurotoxic
					0.831	0.017	Hepatotoxic
					0.773	0.014	Dyspnea
2	Silver ferulate	0.912	0.003	Antiseptic	0.832	0.007	Urine discoloration
					0.785	0.005	Hypercholesterolemic
					0.808	0.034	Shivering
3	Zinc ferulate	0.807	0.004	Antiseptic	0.872	0.014	Diarrhea
					0.829	0.017	Hepatotoxic
					0.806	0.018	Weakness

Selected Phytochemical-Metal Complexes (Synthesis of Ca-Ferulate)



Result of Peak Picking

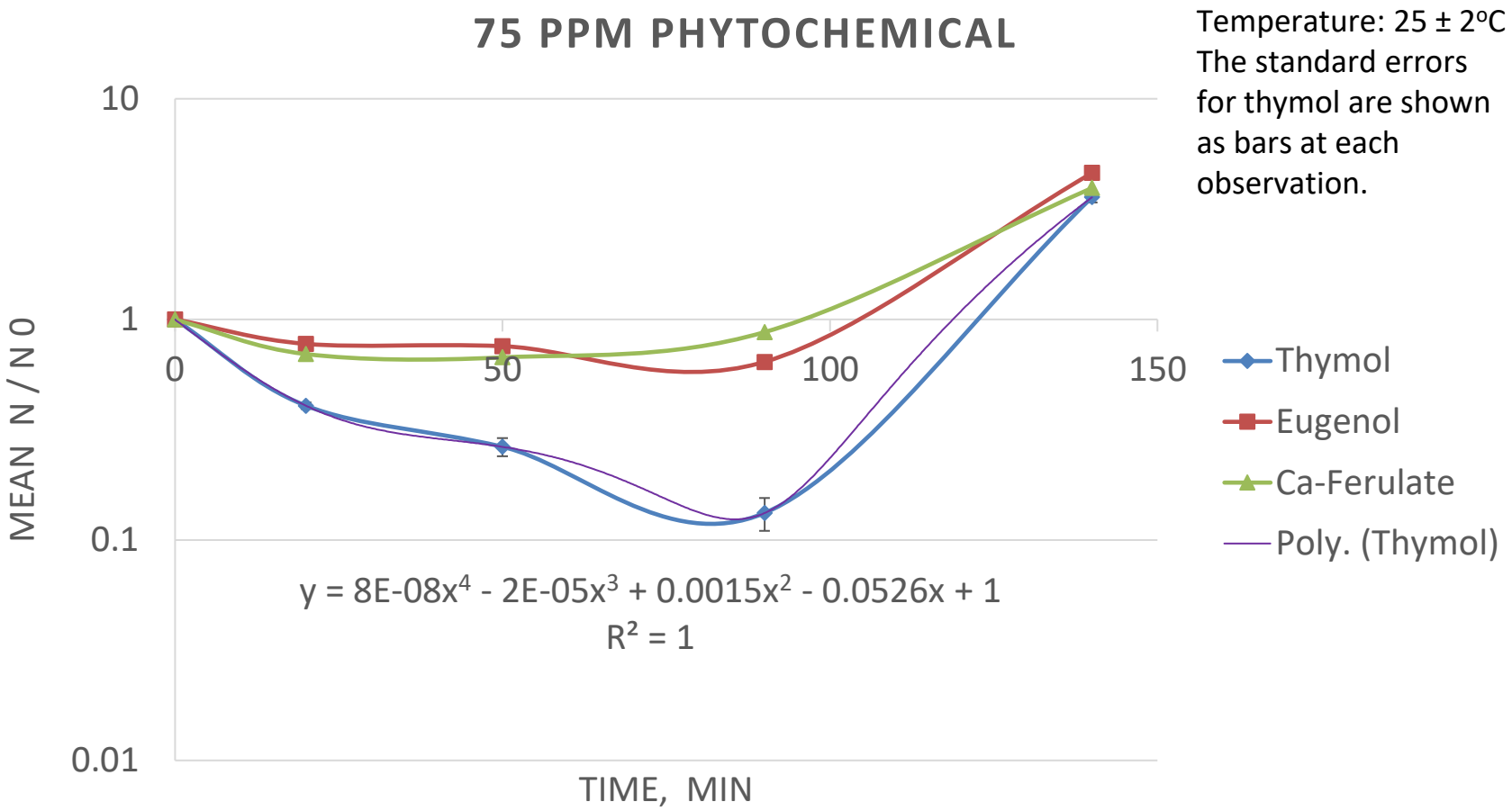
No.	Position	Intensity	No.	Position	Intensity
1	3401.82	31.3492	2	1638.23	20.3557
3	1593.88	20.0722	4	1515.78	7.33987
5	1404.89	7.96784	6	1272.79	8.80375
7	1159.01	29.1641	8	1123.33	26.8114
9	1025.94	38.523	10	979.661	37.428
11	851.418	41.9208	12	819.598	39.4027
13	708.712	47.0386	14	578.54	46.8862

[Comment]
Sample Name
Comment
User
Division
Company Polymer UET

[Measurement Information]
Model Name FT/IR-4100typeA

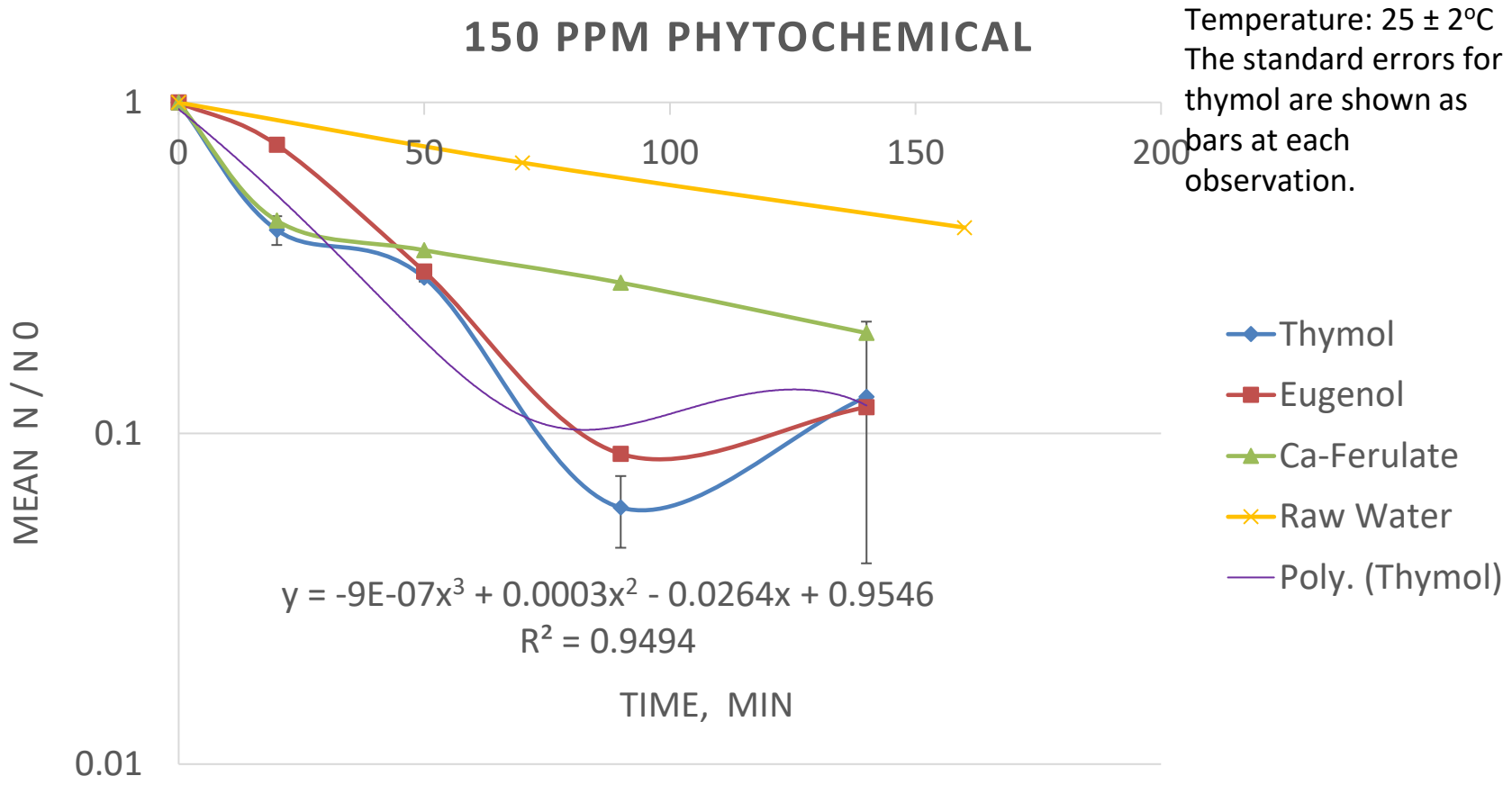
- Direct reaction of ferulic acid with calcium hydroxide yielded calcium ferulate.
- The product residues were subjected to flame test and FTIR analysis.

Disinfection at 75 ppm Phytochemicals



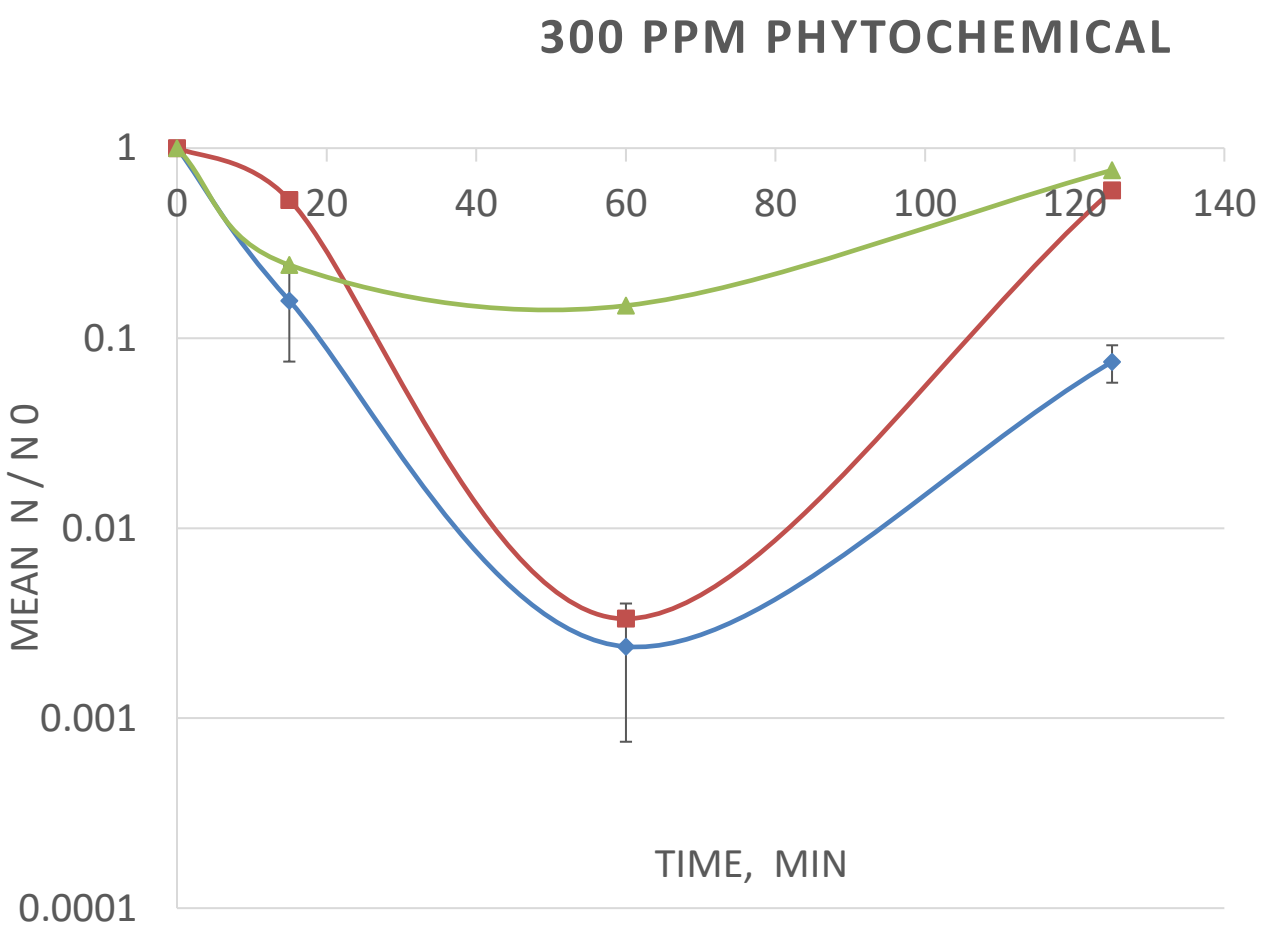
Temperature: 25 ± 2°C
The standard errors for thymol are shown as bars at each observation.

Disinfection at 150 ppm Phytochemicals



Temperature: 25 ± 2°C
The standard errors for thymol are shown as bars at each observation.

Disinfection at 300 ppm Phytochemicals



Temperature: 25 ± 2°C
The standard errors for thymol are shown as bars at each observation.

Disinfection at 300 ppm Phytochemicals

- The results of ANOVA suggest that significant effects of both the disinfectant kind, and time are present.
- The results of Tukey's test also indicate significant differences between the thymol-eugenol and thymol-calcium ferulate.
- There is an optimum time (approx. 1 hour) at which the HPC number decreased to a minimum value. After it, the population began to rise.
- Thymol showed the greatest (of the three phytochemicals), about 2.8 $\log_{10}$, reduction at 60^{th} min interval.

Disinfection at 75, 150, 300 ppm Phytochemicals

- Multiple linear regression were carried out for thymol, and it was found that the following equation (R^2 = 0.956) represented the results:

$$\log\left(\frac{N}{N_0}\right) = -7.940c + 109.876c^2 - 285.228c^3 + 0.111t^3 - 9.918ct + 17.377c^3t^3$$

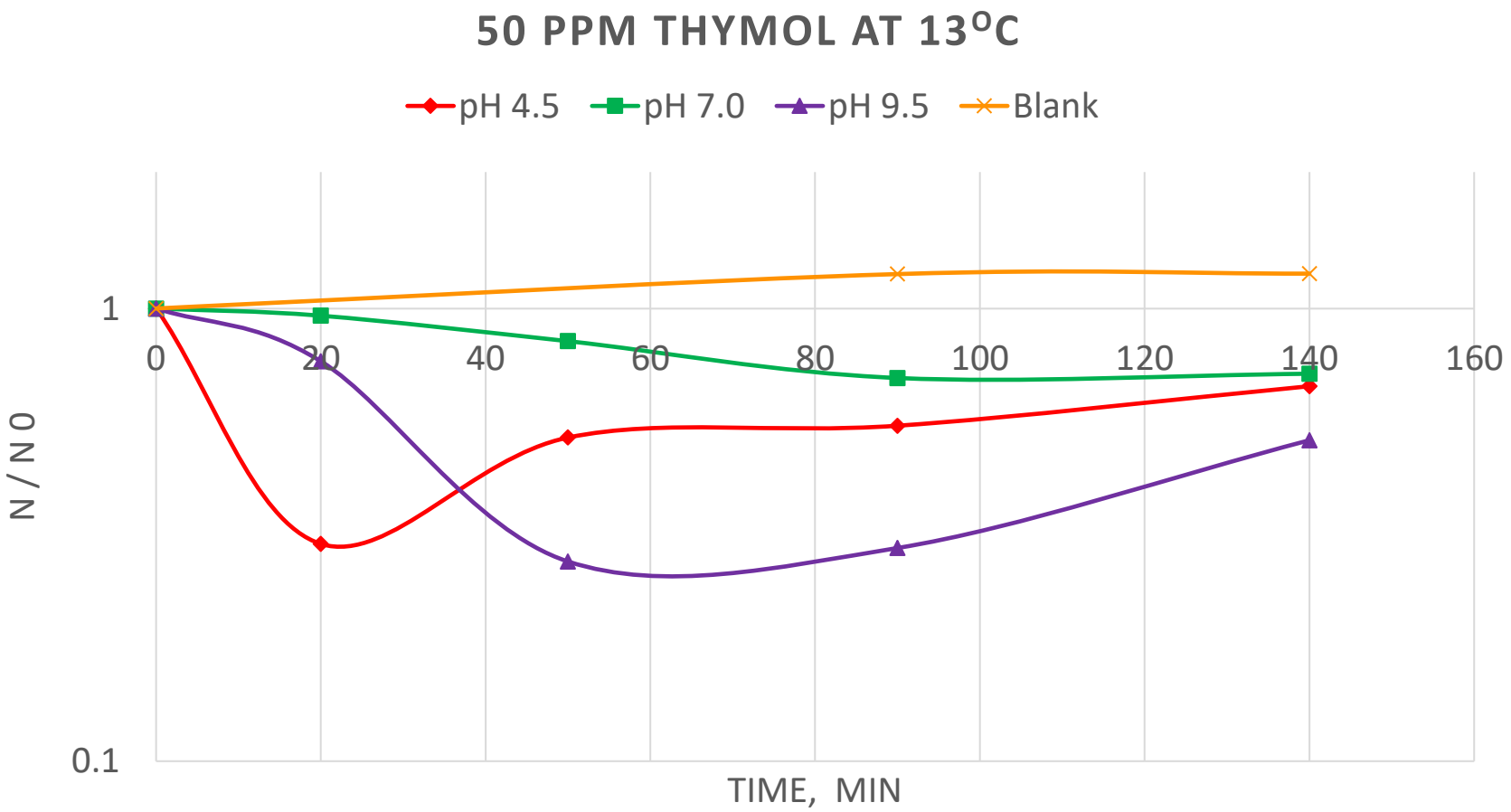
$N =$ Number of HPC microorganisms at time t.

$N_0 =$ Number of HPC microorganisms at initial time.

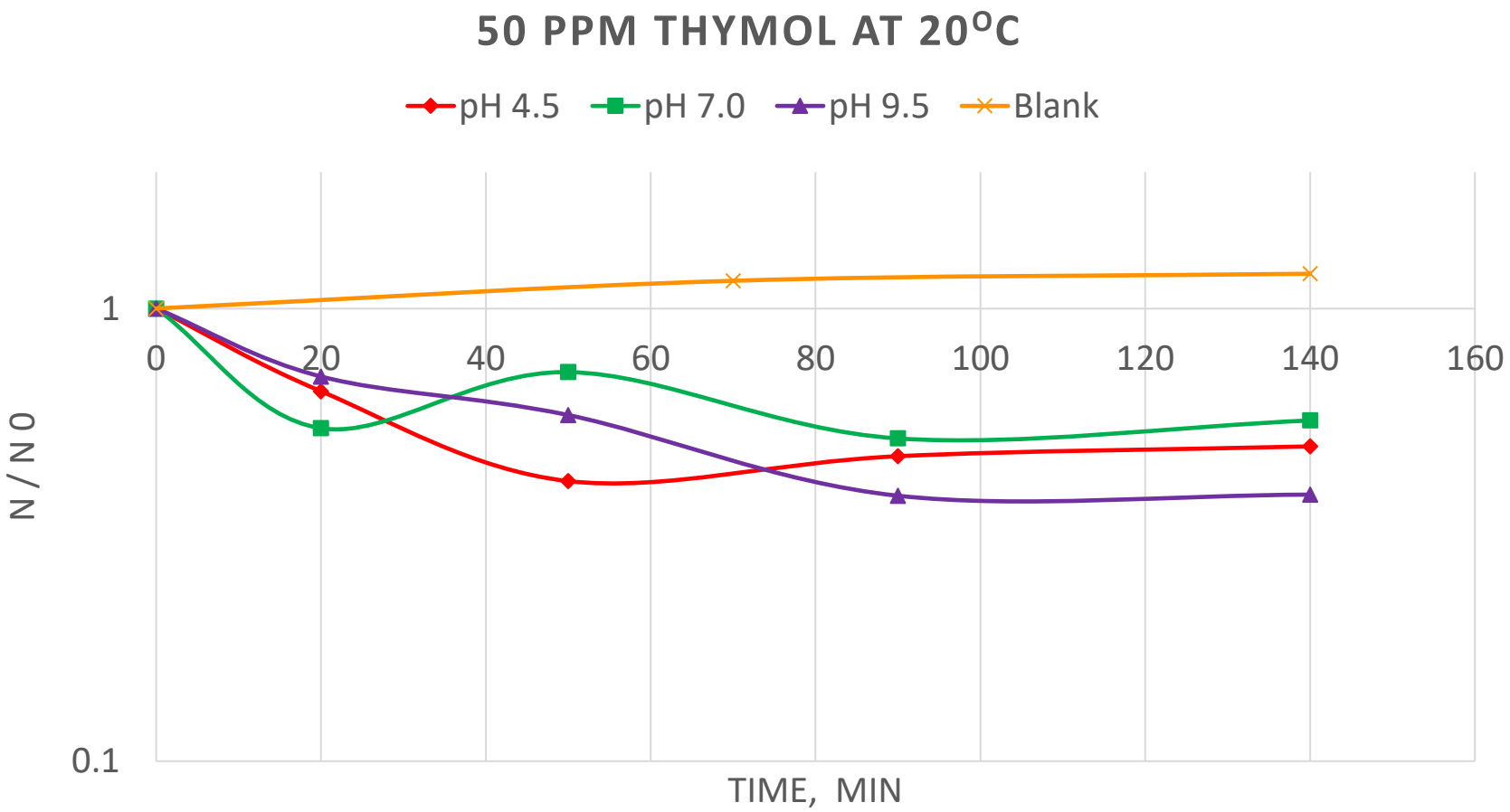
$t =$ Time the disinfectant has been in sample (hours).

$c =$ Initial concentration of disinfectant in sample (g/L).

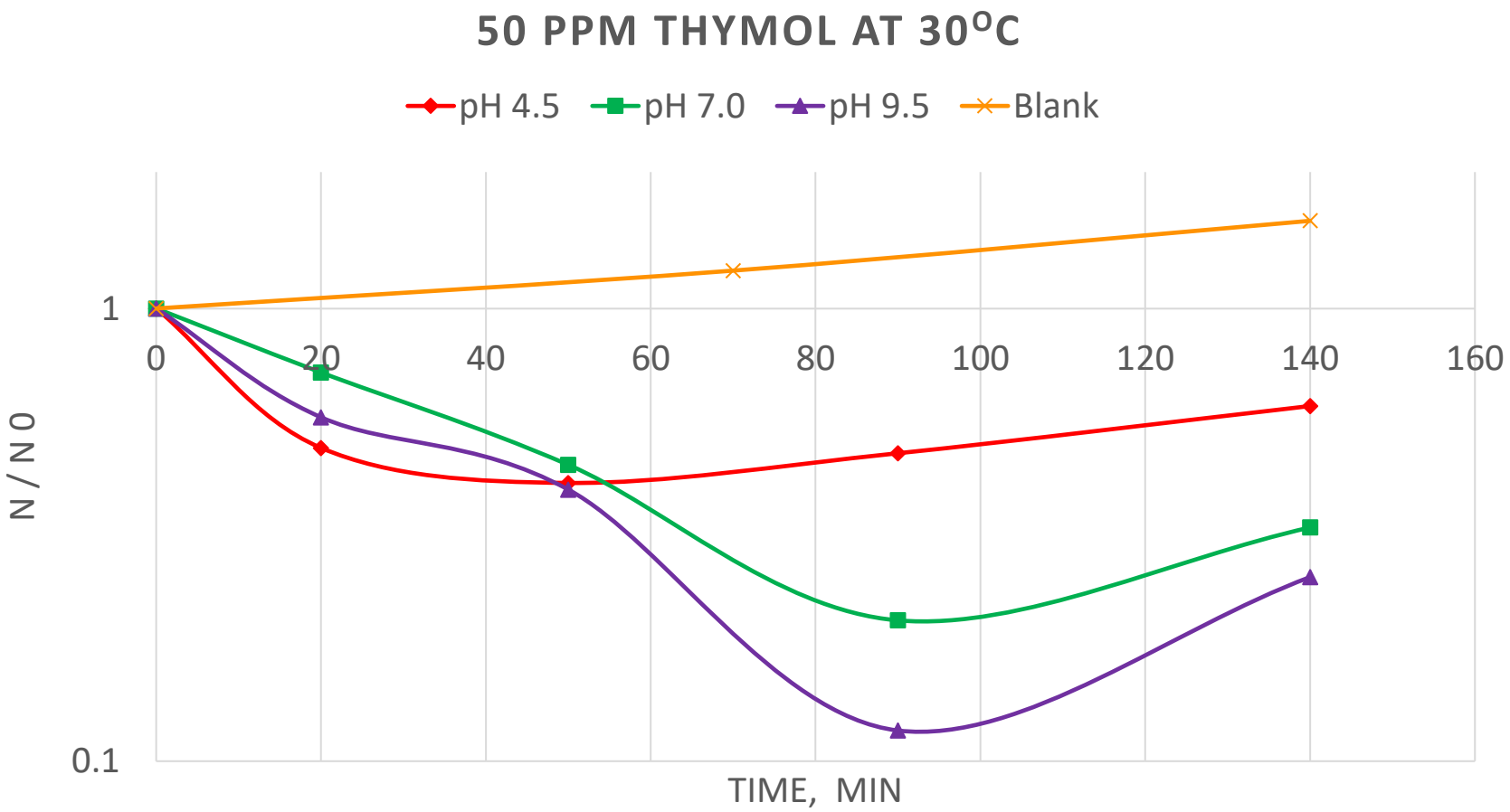
Temperature and pH Effects



Temperature and pH Effects



Temperature and pH Effects

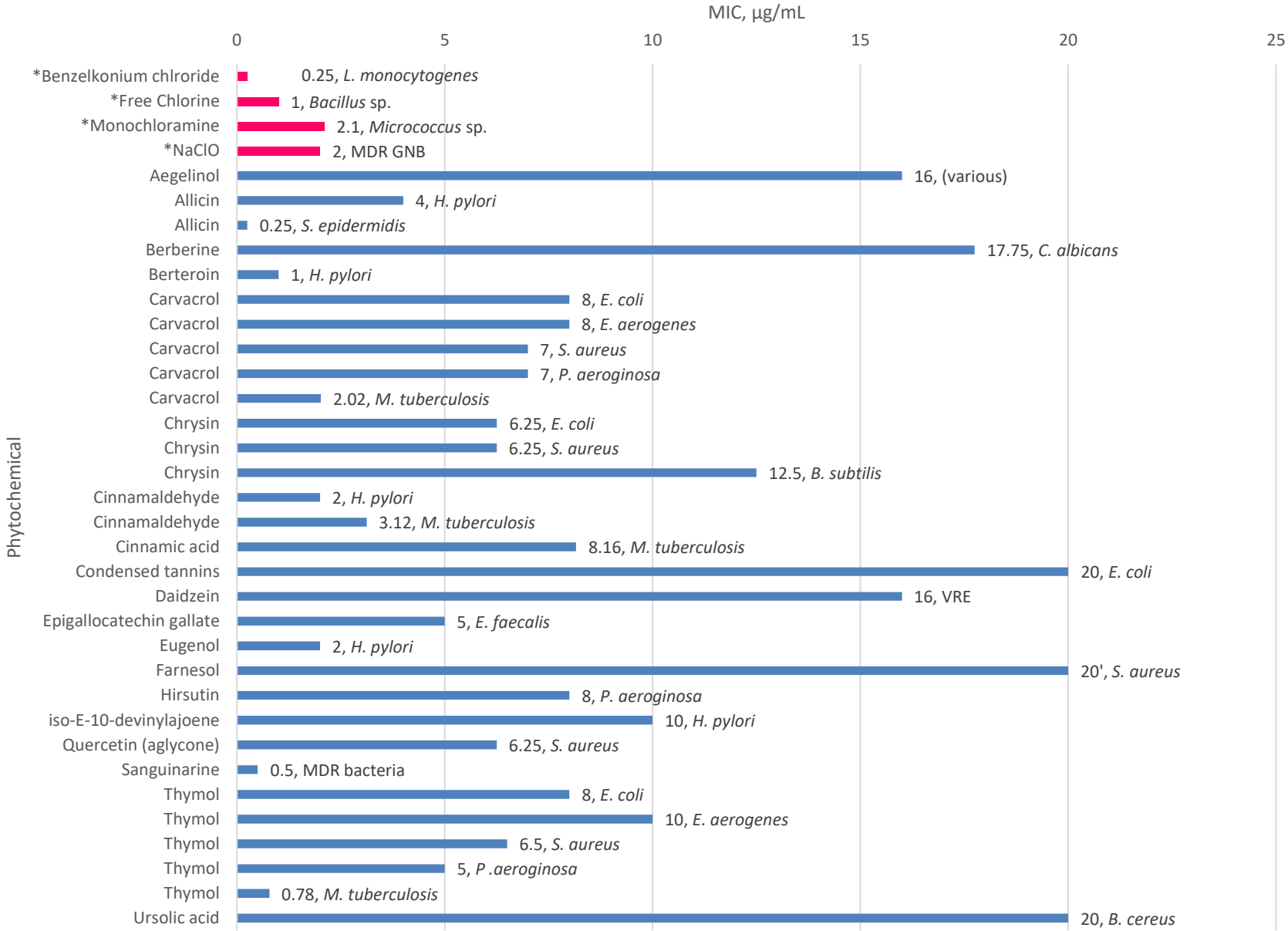


Temperature and pH Effects

- Using multiple linear regression results, the following equation was obtained after optimization for 50 ppm thymol (R^2 = 0.855, pH 4.5 to 9.5, and temperature 13 to 30°C):

$$\log\left(\frac{N}{N_0}\right) = -0.042T + 0.004T^2 - 7.83 \times 10^{-5}T^3 + 0.149t^2 - 0.010Tt - 0.054(pH)t + 0.001(pH)^2t^2$$

  - N = Number of HPC microorganisms at time t.
  - N_0 = Number of HPC microorganisms at initial time.
  - t = Time the phytochemical has been in sample (hrs.).
  - T = Temperature of water (°C).


MIC, µg/mL
0
5
10
15
20
25
Phytochemical
*Benzelkonium chlroride
0.25, L. monocytogenes
*Free Chlorine
1, Bacillus sp.
*Monochloramine
2.1, Micrococcus sp.
*NaClO
2, MDR GNB
Aegelinol
16, (various)
Allicin
4, H. pylori
Allicin
0.25, S. epidermidis
Berberine
17.75, C. albicans
Berteroin
1, H. pylori
Carvacrol
8, E. coli
Carvacrol
8, E. aerogenes
Carvacrol
7, S. aureus
Carvacrol
7, P. aeroginosa
Carvacrol
2.02, M. tuberculosis
Chrysin
6.25, E. coli
Chrysin
6.25, S. aureus
Chrysin
12.5, B. subtilis
Cinnamaldehyde
2, H. pylori
Cinnamaldehyde
3.12, M. tuberculosis
Cinnamic acid
8.16, M. tuberculosis
Condensed tannins
20, E. coli
Daidzein
16, VRE
Epigallocatechin gallate
5, E. faecalis
Eugenol
2, H. pylori
Farnesol
20', S. aureus
Hirsutin
8, P. aeroginosa
iso-E-10-devinylajoene
10, H. pylori
Quercetin (aglycone)
6.25, S. aureus
Sanguinarine
0.5, MDR bacteria
Thymol
8, E. coli
Thymol
10, E. aerogenes
Thymol
6.5, S. aureus
Thymol
5, P .aeroginosa
Thymol
0.78, M. tuberculosis
Ursolic acid
20, B. cereus

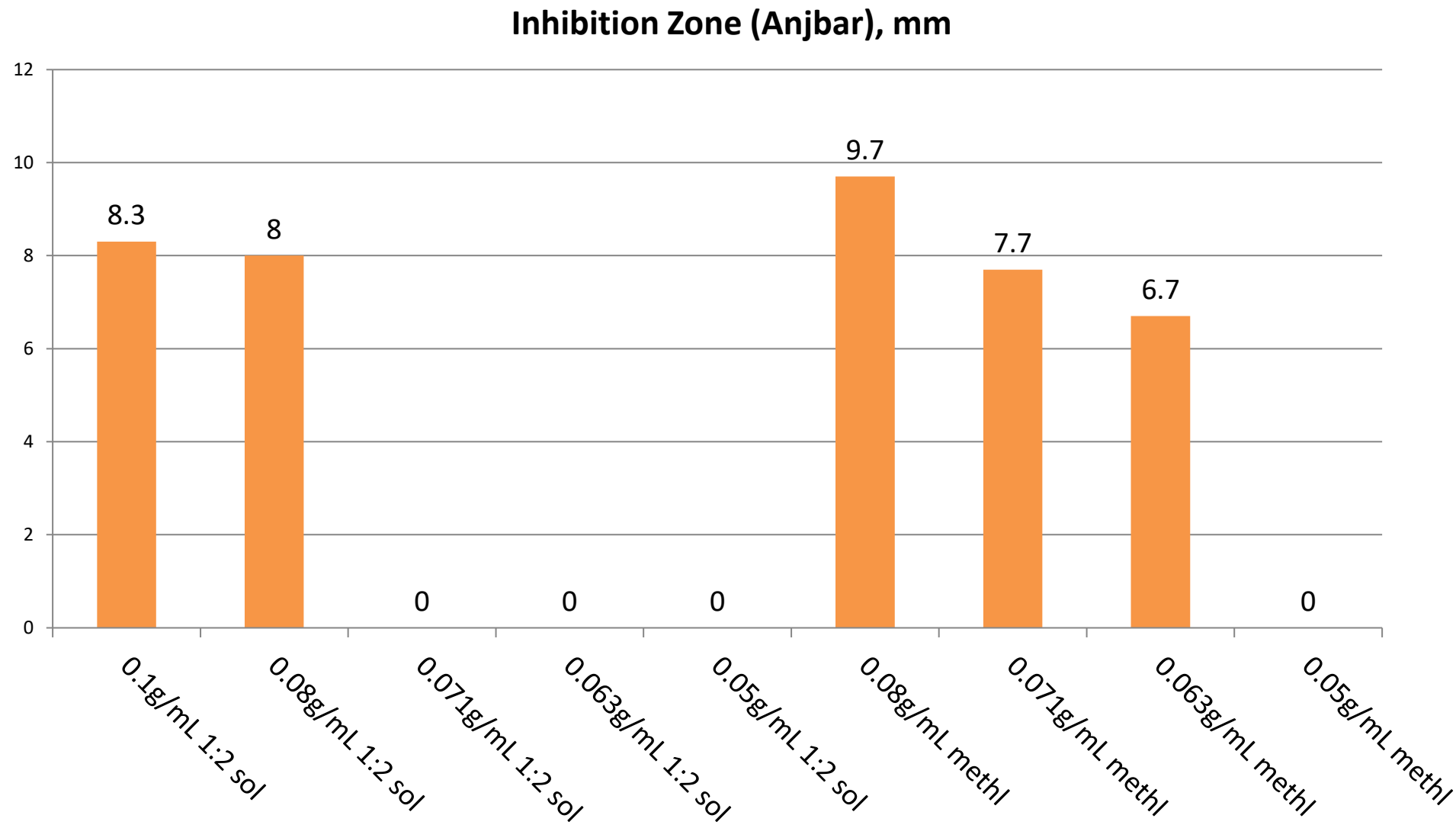
Selection of Suitable Herbs

- About 150 plant species [14-16] were studied.
- The extracts were prepared by macerating [17, 18] for four days exactly 15 g ground sample herb in methanol : water solvent.
- Portions of the extracts were dried for approx. 30 h in total, and solutions of known measurements were also prepared in pure methanol for all herbs.
- Following list of herbs was prepared:

Selected Herbs

Botanical Name	**English Name**	**Urdu & Other Names**	**General Location**	**Parts Used**
Amomum subulatum	Black cardamom	Hull siyaah (*Pers*)	Pakistan, India	Fruit
Colebrookea oppositifolia	Indian squirrel tail	Bansa siah, dhusure	Subcontinent	Bark, leaf
Commiphora opobalsamum	Balsam of Gilead, Arabian balsam tree	Balsaan-e-ajluni (*Arab*)	Both sides of red sea, Saudi Arabia, Yemen, Oman, Egypt	Aerial
(Cyperus articulatus) (Cyperus pertenuis)	A sweet-smelling grass. Papyrus sedges (*Cyperus* spp.)	Naagar motha. Saad (*Arab*, *Cyperus* spp.)	Bangladesh, Burma	Rhizome
Ipomea turpethum (Operculina turpethum)	Turpeth, foe vao, St. Thomas lidpod	Trivrit, nisoth (*Hind*)	India	Leaf
Polygonum viviparum	Alpine bistort	Unjwar, anjbar	Subcontinent	Root
Ptychotis ajowan (Trachyspermum ammi)	Bishop's weed, ajowan, carom	Ajwain	South Asia	Fruit pods
Saussurea lappa	Snow lotus, kuth root, Arabian costus, costus	Koth, kuth, qust, qust shirin	Himalayas	Root

Kirby-Bauer [19] Antibacterial Activity of *Polygonum viviparum*



Conclusions

- With so many antimicrobial phytochemicals being discovered, there is a need for a systematic study to establish their role in water disinfection applications.
- Allicin, berteroin, carvacrol, cinnamaldehyde, eugenol, sanguinarine, and thymol were identified as strong antimicrobials. The results of *in-silico* analysis were in correspondence to the experimental tests.
- In experiments performed using thymol, eugenol, and calcium salt of ferulic acid, thymol was the most effective disinfectant.
- Phytochemical-metal complexes should be experimented for validating the notion of their stronger disinfection capabilities.
- Antimicrobial herbs are not good for use in disinfection of drinking water.

Relevant Research Work

- Wali H. and Ahmed M. M., "Water Disinfection with Plant Products", *Advances in Environmental Technology*, (Article in Press), July 2023. doi: 10.22104/AET.2023.5821.1605
- Wali H. and Zafar M., "Selection Process of Phytochemicals and Efficacy of Thymol, Eugenol and Calcium Ferulate on Heterotrophic Plate Count Bacteria in Water", *J. Chem. Soc. Pak.*, Vol. 41, No. 6, April 2019.
- Wali H., (Ph.D. Thesis) "*Potentiality of a few selected phytochemicals and their metal complexes in drinking water disinfection*", August 2000, University of Engineering and Technology, Lahore.

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12. www.ncbi.nlm.nih.gov/
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THANK YOU