A PROJECT REPORT

ON

SCREENING OF NATURAL COMPOUNDS AS POTENT DISINFECTANTS

Submitted to

Department of Biotechnology and Bioinformatics

Jaypee University of Information Technology, Solan

In the partial fulfillment for the degree of

M. TECH INTEGRATED BIOTECHNOLOGY

By

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Under the supervision of

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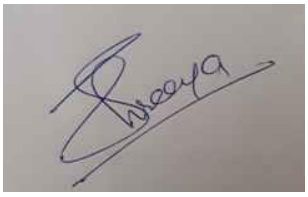
DECLARATION

I hereby declare that the work reported in the M. Tech integrated thesis entitled **"Screening of natural compounds as potent disinfectant"** submitted at **Jaypee University of Information Technology, Waknaghat, India**, is an authentic record of work done by me (Shreeya Agrawal) for the period of July 2020-May 2021 carried out under the supervision of **Dr. Jitendraa Vashistt, Associate Professor**. I have not submitted this work elsewhere for any other degree or diploma.



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Date: 24 May 2021

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Certificate

This is to certify that the work titled "Screening of natural compounds as potent disinfectant," submitted by "Shreeya Agrawal, roll number- '161841' in partial fulfillment for the award of the degree of Masters of Technology Integrated (M. tech integrated) of Jaypee University of Information Technology, Waknaghat has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diplomas.

Signature of supervisor

Date: 24th May 2021

Name of supervisor- Dr. Jitendraa Vashistt

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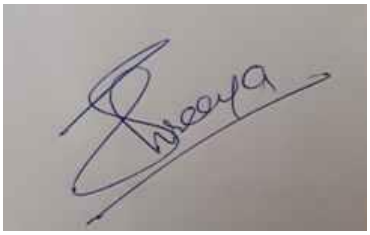
Acknowledgements

The work carried out in this report is an outcome of continual work over a period of time and intellectual support from various sources. It is therefore almost impossible to express adequately the debts owed to many persons who have been instrumental in imparting this work a successful status.

I take this opportunity to thank my esteemed mentor and supervisor Dr. Jitendraa Vashistt, Associate professor of the Department of Biotechnology and Bioinformatics, JUIT, for lending me stimulating suggestions, innovative quality guidance and creative thinking. His practicality, constructive criticism, constant encouragement and advice helped me in all stages of the project. His scientific views and scientific approach will always be the source of motivation for me. I am grateful to him for the support, he provided me in doing things at my pace and for being very patient with my mistakes.

I express my heartful thanks to the Head of Department, Dr. Sudhir Kumar Syal for providing me, with the opportunities of doing this B. Tech project. I would also like to thank my PhD scholars, Ms. Monika Chaudhary who has always been a constant source of inspiration and helped me in numerous ways.

I would also express my gratitude towards my family and my friends for their support throughout the execution of this study. Without their support and encouragement, this journey might have been difficult. Without you all and your support, I wouldn't be here.



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Date: 24 May 2021

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Abbreviations

μg	microgram
mL	milliliter
hrs	hours
HCAI	Healthcare associated infections
FDA	Food and Drug Administration
EPA	Environmental Protection Agency
QAC	Quaternary Ammonium Compounds
IC	Inhibitory concentration

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Chapter 1:
INTRODUCTION

1. INTRODUCTION:

Antiseptics is made from two Greek words "anti" and "septikos", where anti holds the meaning of against, while septikos means rotting or decay. According to *Oxford* English Dictionary, it was first time used in English as an adjective as well as noun in 1751 in *Gentleman's Magazine*, and myrrh, sea salt and acids were regarded as antiseptics. In 1774, antiseptic power of nitrous oxide was remarked by Priestley. And the term Disinfectant came around 17th century [AM Patterson, *et al*, 1932].

Antiseptics and disinfectant have been an unreplaced part of infection control practices in hospitals and even households. In the recent times the importance of sanitation has been increased widely. They have played a magnificent role in prevention of nosocomial infections as well [Larson, *et al*, 1991]. Increased microbial infections and rate with which they are emerging resistant, have increased the use of antiseptics and disinfectants. They are being used on a variety of hard-surface and topical applications. Antiseptics are the compounds which are being used on living cells while disinfectants are the compounds which are being used on hard surface. But a problem which has been noticed over a period of time, is the selection of disinfectant, as different disinfectant reacts differently against different pathogens [Russsell, *et al*, 1996].

From the last few years and in the recent times, the use of alcohol-based disinfectants has been increased tremendously, giving a vision to alternative to attain good hand de-contamination. In the hospitals, apart from use of disinfectants in mopping, cleaning the tables, instruments, advantage of soap and water has also been applied while transiting from one patient to another. But with the time moving, alcohol-based disinfectants have been acting as a suitable alternative of hand washing, especially in the areas where there may not be proper handwashing facilities. While at home, disinfectants being used in regular moppings and cleaning along with regular hand wash as a part of sanitization. But in the recent times, every other person is aware and indeed a user of hand sanitizers. It is so because hand hygiene is one of critical factor in controlling health care-acquired infections i.e., HCAIs [Boyce, *et al*, 2002] as hands may easily become an intermediate in transferring the contamination with transient micro-organisms in the health care.

Sanitizers are also being used in the food industry along with the disinfectant. Disinfectant is used to destroy or inactivate specific fungi or bacteria or both which are infectious irreversibly. These do not affect the spores present on the hard surfaces. Whereas sanitization means to decrease the levels of micro-organisms which are of public health importance to a safe level, based on the parameters already defined, that too keeping the quality and safety of product as a priority.

Despite being said, disinfectants are not being used on skin, and but used, and they do have bad influence on living cell, as it comes in contact with it even for microseconds. Thus, disinfectants can definitely cause serious problems if mishandled. It can cause eye and skin irritation, damages liver as ethanolamine is present in some disinfectants, which adjusts pH of disinfectant to keep it stable, and when it's been exposed in higher doses,

harms kidney and ultimately cause liver disease. Even inhaling of disinfectants can cause respiratory problems. And if over-inhaled, can cause headaches and dizziness. Its ingestion can cause nausea, vomiting. Disinfectants containing butane, which is a highly flammable compound, and is used to spray out the product out of the container, if accidentally consumed or inhaled too much, can create severe cardiac problems.

The main concern is biocides, which according to European legislation are the chemical substances or it can be micro-organism used to harm some other harmful pathogen, and is a broader term including disinfectants, preservatives, pesticides etc. within it, may stop being an effective measure altogether. According to the researchers and FDA, biocides need to be measured thoroughly in the near future, because if any strong resistance arise, tables can turn and instead of being a useful compound, it can be imposed as threat as well. With the increase of side effects of chemical disinfectants, we need disinfectants made up of natural compounds or the combination of natural and chemical compounds. There are many natural compounds which are being used in household as antiseptic or disinfectant. Like neem leaves being used to relief the patients from irritation suffering from chicken pox. The best part with the natural compound is they produce the least side effect even if not positive effect. There are two important aspects which needs to be look forward, first one being prevention of disease transmission and secondly, prevention of the infection cause by it. There is a need of enhancing the efficacy of potency of disinfectants towards the population of microbes. [McDonnell G, et al. 1999; 2001; Siddiqui MS, *et al.*, 2013].

Chapter 2:

REVIEW OF LITERATURE

2. REVIEW OF LITERIATURE:

The terms disinfectant and antiseptic are often confused, and used interchangeably. But we need to know the basic differences between them. If this notable difference could be explained by an example, that could possibly be if any person is undergoing a surgery, doctor will clean the patient's body part with antiseptic while the surgical instruments will be cleaned with disinfectants. Plus, antiseptics contains lower concentration of biocides compared to disinfectants. But there are few compounds which can be used as both, like hydrogen peroxide [Simmonds JH, 2011/12].

2.1 Related terms

Biocide- Biocide can be explained as a micro-organism or chemicals which are used to kill, attenuate providing it is harmless or exerting only a inhibiting effect on other organism which is harmful using either chemical or biological mode [Choudhury, *et al*, 2017]. And according to Environmental Protection Agency i.e., EPA, US "it is a wide group of poisonous substances including insecticides, preservatives, pesticides, and disinfectants, used for the control of organisms that are harmful to the human or animal health or that cause damage to natural or manufactured products." Biocides are divided into four groups.

i) Disinfectant/general biocide
ii) Preservative
iii) Pesticides
iv) Other biocides

We have another term antibacterial, which is often confused with antiseptics as both of them are being used on skin or living cells. But the major difference among them is, antibacterial are used against the bacteria or bacterial infection, while antiseptics will prevent the growth of not only bacteria but also virus, fungi etc.

Germicide- Germicide can be defined as antiseptic or disinfectant that can destroy as well as inactivate pathogens like bacteria, fungi, viruses. It is an agent that can be used against germs. Germicide was defined in 1870 with the same meaning [Merriam webster, America's online dictionary, since 1828].

2.2 **History**

Root idea behind antiseptic word is opposing decomposition while with the disinfectant word is getting rid of the infection. Antiseptics are the compounds applied on the skin/ mucous membranes in order to decrease the amount of flora of microbes. Disinfectants are the molecules which are applied on inanimate objects with the aim of killing of microorganisms, although bacterial spores may get spared by them. It is a procedure

which makes an object safe to use, handle or even discard by removing harmful pathogens from it [AM Patterson, *et al*, 1932; DJ Weber, *et al*, 2007].

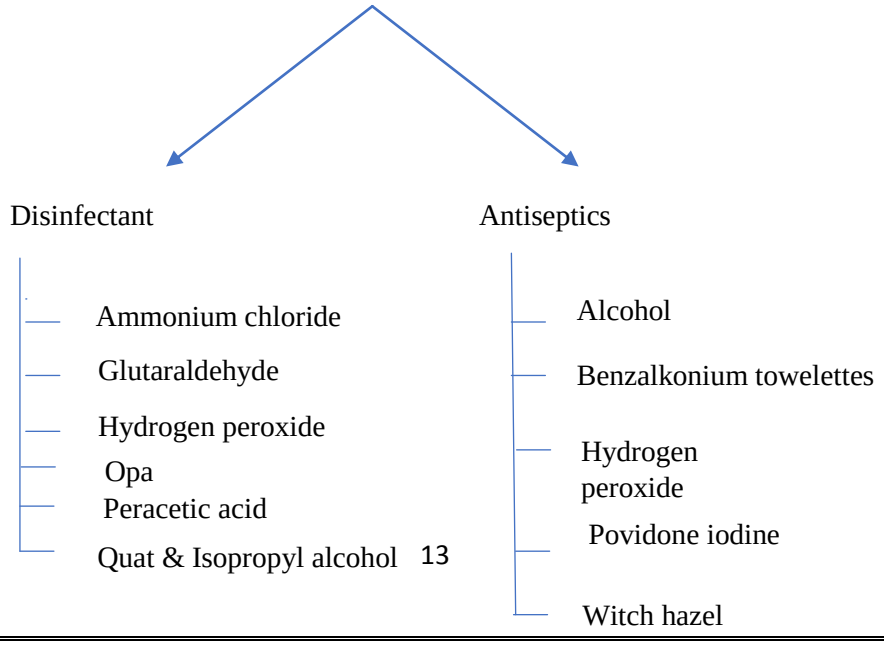
Alcohol like ethanol have been seen registered from very long time their antiseptic use. Since the 19th and 20th century, phenols and hypochlorites have been used and afterwards, quaternary ammonium compounds also came into the scene. And recently, the use of chlorhexidine, peroxygens, silver salts, glutaraldehyde and ortho-phthalaldehyde became wide [AC Abreu, *et al*, 2013].

In surgical practices, alcohol had been used, in the form of wine or some other impure forms in local applications since very long. In 1796, Lowitz, for the first-time prepared alcohol, in which water contained was less than 5%. In 1881, during the monumental study of disinfectants by Koch, he found that when diluted alcohol is compared with absolute alcohol, then former is weak bactericide compared to later one relatively, as indifferent "as sterile water" when used against spores of *Anthrax* [Lowitz, *et al*, 1898-1900; Kch, *et al*, 1881; PB Price, *et al*, 1939].

Vinegar, copper, wine, silver is known to be early disinfectants as well as preservative substances. Mercuric chloride had been known in 4th century as an antiseptic by Arab physicians. At the same time, it was used on ships timber to preserve it, for which patent was also granted in 1832. Condy's crystals or sodium permanganate had been regarded as disinfectant in 1850s. Sterile surgery using disinfectants came into play in 1865 by Lister. He dipped a piece of lint in carbolic acid, which is now known as phenol, solution into the wounds of a 7-years-old boy who had underwent a compound fracture as a cartwheel has passed over his leg at Glasgow Royal Infirmary. After this, there came many discoveries proving to be beneficial against micro-organisms growth on hard surface.

2.3 **Classification**

There are different types of disinfectants and antiseptics. For instance, they have been classified according to compound, manufactured by Medline company production line.



Now, taking into line of its origin, disinfectant can be classified as natural and chemically synthesized disinfectants. Natural disinfectants are the ones which are found in nature with medicinal properties. Their useful part needs to be extracted, processed and needs to be converted in usable form. While chemically synthesized disinfectants are the ones which are made up of chemicals, which can be harmful as well, sometimes, and manufactured in factories. Out of which, some of those chemical products are not sold to common public, it is for lab or special purpose as it can be very harmful and needs specialized supervision.

Disinfectants can be classified of degree of its effectiveness. And on the basis of patient care item, choice of disinfectant agent is done.

i) Low level
- It aims at destroying vegetative spores, along with some viruses and fungi. But it does not harm spores or mycobacteria.
- Chlorine-based products, quaternary ammonium compounds, phenolics, 70-90% alcohol as directed by registered hospital disinfectants by EPA with no tuberculocidal claims.
- In healthcare, it is useful to the noncritical patients care items and surfaces like bed rails, blood pressure cuffs with no visible stain of blood.

ii) Intermediate level
- It aims at destroying mycobacteria, vegetative bacteria, but mostly fungi and viruses, and do not harm bacterial spores.
- Chlorine based products and phenols as directed by registered hospital disinfectants by EPA with tuberculocidal claims.
- In healthcare, it is useful to the noncritical patients care items and surfaces like bed rails, blood pressure cuffs with visible stain of blood.

iii) High level
- It aims at destroying all micro-organisms with an exception of bacterial spores.
- Chlorine, peracetic acid, hydrogen peroxide, phenol, glutaraldehyde, ortho-phthalaldehyde.
- In healthcare, it is useful to the semi-critical patients care re items like vaginal specula, bronchoscopes, gastrointestinal, endoscopes etc [DJ Weber, *et al*, 2007].

2.4 **Chemical disinfectants**

Every chemical disinfectant comes with their own advantages and disadvantages.

i) Alcohols

It's one of the highly effective bactericides, virucidal agent and tuberculocidal agents. It takes mere seconds to kill the microorganism. It possesses the cleansing activity along with being non-toxic and non-irritating. It is non-stainable and can be evaporated without leaving any residue. It is a non-allergic compound. It is inexpensive, easily available and can be easily stored.

At the same time, it is non-sporicidal i.e., cannot kill spores. It's activity against hydrophilic bacteria is also somewhat questionable. It causes irritation in skin a mucous membrane. It does show incompatibility with rubber and plastic materials to some extent. It can be proved to be dangerous, as it is volatile flammable and give rise to inventory and procurement problems. Above all this, it has been not registered as a disinfectant by EPA [McDonnell G, *et al*, 1999; 2001; Siddiqui MS, *et al*, 2013].

ii) Chlorine

It is broad spectrum biocide, a chemical to destroy, the micro-organism or to have control over any harmful pathogen by chemical or biological mode. It holds sanitizing properties with poor toxicity and irritancy. It is also non-staining and colorless compound with an odor which can be tolerated easily. It is a low-cost disinfectant with not so important residues.

Along with this, it causes skin irritation if its contact with skin is prolonged. Its inhalation can also lead to bronchial irritation. It does have caustic effects in general. Caustic effects are the effects of light upon an object through another object via refraction or reflection. It has the capacity to corrode the metal as well as, can deteriorate the product if left standing. It is used as bleach on fabrics. It has co-carcinogenic properties, i.e., chemicals that has the ability to trigger the symptoms of carcinogen production in the case of cancer. Interestingly, this compound has also not been registered as disinfectant by EPA

iii) Formaldehyde

It's has been registered as a disinfectant by EPA. It's also a broad-spectrum biocide. It can be neutralized rapidly and holds very good vapor phase disinfectant paraformaldehyde. It has been found active in organic matter as well. It is relatively expensive and can be bought very easily from the market. It is stable as well, if properly stored.

Above all this, its smell is pungent beyond limit which may become suffocating at times. At the same time, it can cause skin and mucous membrane irritation, along with skin allergies.

iv) Glutaraldehyde

It is broad spectrum antimicrobial compound. It acts better in presence of organic matter in comparison with any other compound like metal, rubber, or any plastic material. It has low volatility and nonflammable. It shows rapid action as disinfectant. EPA has registered glutaraldehyde as disinfectant and sporicide both.

At the same time, it is to be activated, cannot activate on its own. And removal of residual disinfectant requires rinsing as well. It is regarded as slightly and moderately toxic. It is not advised to be used with the carbon steel surfaces. Its prolong use or inhalation causes tissue irritation especially in eyes and mucous membrane, causing severe allergies in skin. It has definite shelf life [McDonnell G, *et al*, 1999; McDonnell G, *et al*, 2001; Siddiqui MS, *et al*, 2013].

v) Iodophors

It is a derivative of iodine and EPA has also registered it as a disinfectant. It is one of the powerful germicides, which is relatively less toxic and causes little irritation. Thus, one can say, it is safe and convenient to use. It can readily dissolve in water and possess a power detergent property. It is colorless and non-stainable compound.

But it does show corrosiveness on some surfaces and is unstable above $54^{o}C$. Satisfying this, it cannot be used in instruments, as it may corrode them. It is comparatively little expensive.

vi) Phenolics

A broad-spectrum antimicrobial compound with good cleansing actions. It can be a good sanitizer or germicide at household. It can easily dissolve with additives like soap. Many formulations for phenolic compounds are available and its coefficient is readily achievable. EPA has registered phenolic compounds as disinfectant.

But it is a poor sporicide, with bad odor. It is a toxic compound which can cause mucous membrane and skin irritation. It can be deactivated by organic matter unlike some other disinfectants which gets activated by organic matter.

vii) Quaternary Ammonium Compounds

EPA has registered this compound as disinfectant. It is an inexpensive compound with a good disinfection property against gram positive microbes. It is readily soluble in water and alcohol and has a pleasant aromatic smell. It has surfactant and detergent properties. It can be readily prepared and used. It can be used in various sanitizing applications. It is nontoxic and non-allergenic.

But at the same time, it is poor against gram negative microbes and also lacks behind in tuberculocidal properties, with no sporicidal activity. It is also ineffective against pseudomonas, hydrophilic viruses, nosocomial infections, which ultimately gives rise to resistant bacterial strains. It can be inactivated by organic matter, gauze, stoppers, cotton etc.

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2.5 Mode of action of chemical disinfectants

i) Alcohol- It works better when used along with water. It causes

- Denaturant the proteins.
- Damages cell membrane.
- Frequent interference in cell metabolism.
- Ultimately, cell lysis [McDonnell G, *et a*, 1999; McDonnell G, *et al*, 2001; Siddiqui MS, *et al*, 2013].

ii) Glutaraldehyde-

- When the target organism is bacterial spores, low concentration of glutaraldehyde can only inhibit the concentration, while high concentrations due to stronger interaction along with the outer layers of cells kills the spores.
- When the target is other non-sporulating bacteria, glutaraldehyde shows powerful association with outer layers of gram- negative and gram0positove bacteria. Besides this it crosslinks different amino acids in the proteins present. It also inhibits the process of transport into the cell.
- When the target organism is fungi, its cell wall is its primary target, it shows postulated interaction with the cell wall's main component chitin.
- When the target organism is mycobacteria, mode of action is not known clearly, but it involves its cell wall.
- When the target organism is viruses, its mode of action also not very clear, but studies show it involves cross-links between protein-DNA and alteration in capsid.

iii) Ionophores-

- Even at low concentration, it has high antimicrobial action.
- It can penetrate into microorganism with a great speed, and targets the main group of proteins, specifically cysteine, methionine which has free Sulphur amino acids, fatty acids and nucleotides. All this leads to the cell death.
- Lipid enveloped viruses are more sensitive towards iodine compared to non-lipid and parvo viruses, as in enveloped proteins, it attacks the surface proteins, as it does in case of bacteria also. This may lead to destabilization of fatty acids present in membrane with unsaturated carbon bonds..

iv) Quaternary Ammonium Compounds (QAC)

- Targets cytoplasmic inner membrane.
- Involves phospholipid bilayers.

But with time, chemical disinfectants have developed resistance for certain pathogens and the amount required of them to be effective has become little high which can be harmful to the animals present around the applicative area. So, as an alternative, natural products solely, or a mixture of natural and chemical disinfectants can be used against the pathogens.

2.6 Natural compounds found in plants

There have been many primary and secondary natural compounds produced by plants, which are being utilized. Primary products like fruits, vegetables, logs etc., while secondary products like flavonoids, alkaloids, tannins etc., are being used by plants as their defense mechanism or to elevate the production of primary products. But along with these, these secondary compounds can be used for disinfectant properties as well [Seigler, *et al*, 1976].

Mode of action of secondary products from plants

i) Flavonoids

- It damages cytoplasmic membrane.
- It inhibits nucleic acid synthesis
- It inhibits the energy metabolism.
- Ex. Kaempfol, quercetin etc., [Ikigai, *et al*, 1993; Mori, *et al*, 1987; Haraguchi, *et al*, 1998; Cushnie, *et al*, 2005].

ii) Alkaloids

- It can intercalate or alkylate DNA and thus,
- It affects the cell division and disrupts membrane of the bacteria.
- It inhibits respiration and enzymes in bacteria.
- Ex. Codeine, scopolamine etc., [Amin, *et al*, 1969].

iii) Tannins

- It complexes protein with covalent and non-covalent interaction.
- It is also capable of complexing with polysaccharide.
- Ex. Chlorogenic acid, catechins etc., [Amin, *et al*, 1969].

iv) Saponin

- It interacts with the sterols, which is found in the membrane of micro-organism, and damages the membrane.
- This results in the leakage of cell content and ultimately, cell death.
- Ex. Squalene, quillaia, etc., [Patra, *et al*, 2009].

v) Terpenoids

- Carbonylation of terpenoids has been reported, increases bacteriostatic activity.
- Ex. Sesquiterpenes, monoterpenes etc., [Griffin, *et al*, 1999; Naigre, *et al*, 1996].

2.7 Natural plants with disinfectant property compounds

India being the one of the major producers of herbs, specially medicinal herbs thus gladly known as "botanical garden of the world" [N. Shariff, *et al*, 2006]. Moreover, in India, more than 80% of population uses home remedy for many day-to days problems [G. Vines, *et al*, 2004]. From applying turmeric with mustard oil in wounds to using neem leaves to sooth the itching and irritation from chicken pox, it is very common in Indian homes, as these do not have any harmful effect, even if they do not have any positive effect. The plants with medicinal value can be in drug development as they are the source of the compounds holding therapeutic esteem. This caliber of medicinal herb can be utilized and molded in wonderful ways. One of which could be using them as disinfectant or combining them with chemical disinfectants, which would be non-toxic or least toxic to living cells, at the same time, lethal to micro-organisms.

1. *Acacia*

   Acacia is a large genus consisting of trees as well as shrubs. Commonly known as acacias or wattles. It belongs to the family of Fabaceae, with subfamily Mimosoideae. It is one of the original as well as a native plant of Australia and Africa, but it is not limited to that are only. Acacia willd is a huge genus consisting of trees, climbers as well as shrubs. *Acacia nilotica* is very common in

India, locally known as babul, babur, kikar. It is medium sized tree with a crown spreading look.

Using GC- mass spectra analysis 3-cyclohexane-1-carboxaldehyde, 2,6,6-trimethyl; oleic acid; globulol; selinene (á); were found as active phytochemical compounds when an aqueous extract of *A. nilotica* was analyzed as shown in table 1. These bioactive compounds were effective against *K. pneumoniae*, *P. aeuroginosa*, *E. coli*, and *P. mirabilis.* These bioactive compounds were found to be showing antimicrobial as well as anti-biofilm activity. Cytotoxicity level in Acacia nilotica has been reported increasing from the conc. of 0.5 µg/mL - 500 µg/mL [Khalid, *et al.*, 2017; Elamary, *et al.*, 2020; Arya, *et al.*, 2019; Khalid, *et al*, 2010; T Kalaivani, *et al*, 2011; A Barapatre, *et al*, 2016].

Table1: Active compounds found among *Acacia nilotica*.

Plant	Active Compound		Reference
Acacia (*A. nilotica)*	i) 3-Cyclohexane-1-Carboxaldehyde, 2,6,6-trimethyl		Khalid, *et al*, 2017; Elamary, *et al*, 2020; Arya, *et al*, 2019; Khalid, *et al*, 2010
	ii) oleic acid	unsaturated fatty acid	
	iii) á-Selinene	sesquiterpenes	
	iv) globulol	alcohol	

2. *Allium sativum*

Allium sativum is one of the species from the family of onion and is used as a food ingredient as well as in traditional medicine. China being the major producer of garlic, and produces around 80% of total world's production.

Majority of the people choose the bulb part which is divided into number of fleshy sections, commonly known as cloves as food due to its spicy and pungent flavor These flavors are actually due to the presence of organosulphur compounds along with allicin and ajoene. The distinct smell which comes from the mouth after consuming garlic, comes due to presence of allyl methyl sulfide.

It is not only the bulb part which is edible, but its leaves and flowers are also used by people in their food habits. The flowers and leaves have milder flavor than the bulb and they are plucked when still immature and tender. Immature garlic is sold as "green garlic" in the market.

It contains bioactive compounds allicin (Diallyl thiosulfonate), diallyl disulfide (DADS), diallyl sulfide (DAS), Diallyl trisulfide (DATS), E/Z- ajoene, S-allyl-cysteine sulfoxide (alliin), S-allyl-cysteine (SAC) as mentioned in table 2. Allicin is the main compound in the garlic, which cannot be not found in raw garlic; but

is rapid produced from alliin, when alliinase act on it. Alliinase activates when garlic is being cut or crushed. Allicin degrdation produces DAS, DADS, and DATS [Butt, *et al*, 2009; WHO monographs, 1999; Harris, *et al*, 2012; Rao, *et al*, 2008; Plants of the world online, 2021; Block, *et al*, 2010; Rahmani, *et al*, 2014; Rahman, *et al*, 2007; Fleischauer, *et al*, 2001; Weber, *et al*, 1992; Khar, *et al*, 2011; Bhaghalian, *et al*, 2005].

Table 2: Active compounds found among *Allium sativum*

Plant	Active Compound	Reference
Garlic (*Allium sativum*)	Allicin (Diallyl thiosulfonate)	Butt, *et al.*, 2009; WHO monographs, 1999; Harris, *et al.*, 2012; Rao, *et al.*, 2008; Plants of the world online, 2021; Block, *et al.*, 2010; Rahmani, *et al.*, 2014; Rahman, *et al.*, 2007; Fleischauer, *et al*, 2001; Weber, *et al.*, 1992; Khar, *et al.*, 2011; Bhaghalian, *et al.*, 2005
	Diallyl-sulfide (DAS)	
	Diallyldisulfide (DADS)	
	Diallyltrisulfide (DATS)	
	E/Z- ajoene	
	S-Allylcysteine (SAC)	
	S-allylcysteinesulfoxide	

3. *Juglans regia*

Walnuts can be nuts from any tree from the genus of *Juglans* genus, from the family of Juglandaceae. *Juglans regia* is a commonly used. It is not considered as true botanical nut, as only the drupe part is the edible seed part in it. After the edible seed ripens, and the shell discards, the resulting part can be used in garnishing or as a snack.

The bioactive compounds found in this are in kernel part are p-hydroxybenzoate, vanilliate, gallate, rutin, naringin, quercetin-3-D-glucoside, syringic acid. The dried bark of Juglans regia is known to be dandasa. *Juglans nigra*, commonly known as black walnut is a high valued tree in US and holds multiple of qualities like anti-oxidant, anti-microbial, anti-inflammatory etc. *Juglans regia* have the antibacterial, anti-biofilm properties against *M. morganii*, *P aeruginosa*, *A. baumannii*, *H. influenzae*, *K pneumoneae*, and *S. aureus*. a combination was found to be very effective. It was reported that between 0.226 to less than 0.5 mg/mL concentration in methanol have been proved to be cytotoxic against cancer cells [“Walnut; Agriculture- Transport Information Service”, 2010; Khalid,

et al, 2020; Faraz, *et al*; Ho KV, *et al*, 2018; Faraz N, *et al*, 2012; M Salimi, *et al*, 2012; M Carvalho, *et al*, 2010].

Table 3: Active compounds found among *Juglans regia.*

Plant	Active Compound		Reference
Walnut (*Juglans regia)*	i) vanillic acid	benzoic acid	"Walnut; Agriculture-Transport Information Service", 2010; Khalid, *et al*, 2020; Faraz, *et al*; Ho KV, *et al*, 2018; Faraz N, *et al*, 2012
	ii) p-hydroxybenzoic acid	benzoic acid	
	iii) gallic acid	phenolic acid	
	iv) quinic acid	carboxylic acid	
	v) syringic acid	deriavative of gallic acid	
	vi) epicatechin gallate	ester	
	vii) rutin	sophorin	
	viii) naringin	flavonoid glycoside	
	ix) quercetin-3-D-glucoside	glucoside	

4. *Camellia sinensis*

Botanical name of green tea is *Camellia sinensis* belonging to the family of Theaceae. It is different from normal black oolong teas as their buds and leaves have undergone different withering and oxidation process. It was originally originated in China, and now its manufacturing and production has been spread to other countries of East Asia as well.

It contains a number of polyphenols, from which catechins (epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin (EC), epicatechin gallate (ECG), which may act as anti-bacterial. The activity of polyphenols is good against gram positive bacteria.

A combination of dandasa and green tea was found to be useful. dandasa with 6.2mg/ml and green tea at 12.5 mg/ml is said to have good antibiofilm property against *Streptococcus mutan.* Dandasa with 12.5mg/ml and green tea at 3.1 mg/ml holds good anti biofilm property against *E. coli* [Khan, *et al*, 2013; Faraz N, *et al*, 2012].

Table 4: Active compounds found among *Camellia sinensis.*

Plant	Active Compound	Reference
Green tea (*Camellia sinensis*)	i) epigallocatechin (EGC)	Khan , *et al*, 2013; Faraz N, *et al*, 2012
	ii) epigallocatechin gallate	
	iii) epicatechin gallate	
	iv) epicatechin	

5. *Syzygium aromaticum*

Clove oil or oil of clove is one of the essential oil, and taken out from *Syzygium aromaticum*, clove plant. It is very used in aromatherapy, as flavoring agent in food and medicines. But along with this it has been observed that clove oil has been observed to affect the quorum sensing pathway of bacteria, which ultimately breaks its further regulations in *Pseudomonas aeruginosa* bacteria.

Using bioassay-directed chromatographic fractionation method, different compounds have been identified in clove oil as- 8-C-β-D-glucopyranoside, biflorin, kaempferol, ellagic acid, 5,7-dihydroxy-2-methylchromone, rhamnocitrin, myricetin, gallic acid and oleanolic acid..

Table 5: Active compounds found among *Clove oil.*

Plant	Active Compound	Reference
clove oil	5,7-dihydroxy-2-methylchromone	"Clove" drugs.com 3 july 2020; clove medlines; Cai, *et al.*, 1996; Husain, *et al.*, 2013; Husain, *et al.*, 2013; Khan, *et al.*, 2009
	8-C-β-D-glucopyranoside	
	biflorin	
	kaempferol	
	rhamnocitrin	
	myricetin	

	gallic acid	
	ellagic acid	
	oleanolic acid	

6. *Origanum vulgare*

   Origanum vulgare (family Lamiaceae) is a herb and so its plant parts like leaves are used as flavoring agent. Interestingly, it is more valuable or flavorful in dried form compared to fresh leaves.

   It has high phenolic content, which helps in its being an anti-oxidant property imparting plant. 5 major phenolic compounds have been identified: rosmarinic acid, caffeicacid, protocatechuic acid, couramins. Thymol and carvacrol have also been reported in this. The critical conc. of phenol was sufficient to obtain antioxidant activity as it reaches to saturation effect, and increasing more phenol to it, does not elevate its response. It has also been observed effective against *H. pylori*, which is responsible for ulcers in human. IC_{50} against. [Mockute , *et al.*, 2001; chun, *et al.*, 2005; Baratta, *et al.*, 1998;].

Table 6: Active compounds found among *Oregano.*

Plant	Active Compound	Reference
Oregano	i) rosmarinic acid	Mockute , *et al.*, 2001; chun, *et al.*, 2005; Baratta, *et al.*, 1998; "American: Oregano" Collins Dictionary.
	ii) caffeic acid	
	iii) coumaric acid	
	iv) protocatechuic acid	
	v) quercetin	
	vi) Thymol	
	vii) carvacrol	

7. *Aegle marmelos*

   Aegle marmelos (monotypic genus Aegle) is known as Bael plant in India is a found abundantly in Asia. A genus is known as monotypic genus when a single species and genus are described simultaneously. More than 30 compounds have been reported from this plant as mentioned in the table 1. From root to stem, every part of this plant is proved to be useful and used for treating either one of the problems face by human society. It can be used against cancer, cardiovascular, gastrointestinal disorders. It also acts as antimicrobial, anti-spermatogetic, anti-inflammatory, anti-hyperlipidaemic, anti-oxidant, antiulcer, antidiabetic, anticancer. It has also been reported useful for skin and eyes infection. From the extracts of bael leaves, IC_{50} amount was found after 24 hrs and 48 hrs incubation as 50 and 72 μg/mL respectively [Mujeeb, *et al.*, 2014; Badal, *et al.*, 2002; Chew, *et al.*, 2009; Gupta, *et al.*, 2004; Kamalakkannan, *et al.*, 2205; Arul, *et al.*, 2005;

Jagetia, *et al.*, 2005; Kamalakkannnan, *et al.*, 2003; Rajadurai, *et al.*, 2005; Sabu, eta l, 2004; Saradha, *et al.*, 2010; Sur, *et al.*, 1999; Yen, *et al.*, 1993; K Kulprachakarn, *et al*, 2020].

Table 7: Active compounds found among *Aegle marmelos.*

Plant	Active Compound			Reference
Aegle marmelos (Bael)	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Flavonoid fraction	Antimicrobial, anti-inflammatory, antiproliferative	Mujeeb, *et al.*, 2014; Badal, *et al.*, 2002; Chew, *et al.*, 2009; Gupta, *et al.*, 2004; Kamalakkannan, *et al.*, 2205; Arul, *et al.*, 2005; Jagetia, *et al.*, 2005; Kamalakkannnan, *et al.*, 2003; Rajadurai, *et al.*, 2005; Sabu, eta l, 2004; Saradha, *et al.*, 2010; Sur, *et al.*, 1999; Yen, *et al.*, 1993
	1-Butanol, 3-methyl-, acetate	Alcohol	Anti-microbial	
	2,3 Dioxabicyclo2.2.2oct-5-ene, 1-methyl-4-(1-methylethyl)-(Limonene dioxide 1)	Terpene	Anti-microbial	
	Bicyclo3.1.1heptane-2,3-diol, 2,6,6-trimethyl (2,3-Pinanediol)	Terpene	Anti-microbial	
	2-Cyclohexen-1-one, 4-hydroxy-3-methyl-6-(1-methylethyl)-		antibacterial	
	1-Dodecanol	long-chain fatty alcohol	antibacterial	
	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl (BHT)		Antimicrobials, antioxidant	
	Benzoic acid, 4-ethoxy-, ethyl ester	Aromatic acid ester	Preservative	
	2-Propanol, 1,1′-(1-methyl-1,2-ethanediyl)bis(oxy)bis-(Tripropylene glycol)		Anti-microbial	
	1-Tetradecanol, acrylate	Fatty acid esters	Anti-inflammatory,	
	1,3,4,5-Tetrahydroxy-cyclohexanecarboxylic acid (Quinic acid)	Aromatic acid	Anti-microbial	
	Tetradecanoic acid (Myristic acid)	Fatty acid	Anti-fungal, cancer prevention,	

	2(4H)-Benzofuranone 5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl	Triterpene	antimicrobial	
	1-Heptadecanol (1-Eicosanol)	Aliphatic alcohol	Antimalarial, antifungal, Antioxidant	
	1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)- (1-Phellandrene)	Monoterpene	Antibacterial	
	1,6-Octadiene, 7-methyl-3-methylene (beta.-myrcene)	Monoterpene	Antibacterial	
	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester (Cinnamic acid, 4-hydroxy-3-methoxy-, methyl ester)	Aromatic methyl esters	Antimicrobial, antioxidant, antiviral	
	Pentadecanoic acid	Fatty acid	Antibacterial	
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	Diterpene	Anti-microbial, anti-cancer,	
	hexadecanoic acid, methyl ester (Palmitic acid methyl ester)	Fatty acid methyl ester	Anti-oxidant, anti-androgenic, hemolytic,	
	Pentadecanoic acid	Saturated fatty acid	Anti-microbial	
	9-Octadecenoic acid	Unsaturated fatty acid	antibacterial	
	Heptadecanoic acid	Saturated fatty acid	Antimicrobial	
	9,12,15-Octadecatrienoic acid, methyl ester (Linolenic acid, methyl ester)	Fatty acid methyl ester	Antimicrobial Anti-cancer hepato-protective, Anti-androgenic	
	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl (Phytol isomer)	Diterpene	Anti-microbial Anti-cancer Di-uretic	
	Cis-9-Hexadecenal	Aldehyde	Antimicrobial	

	Octadecanoic acid (Stearic acid)	Fatty acid	Antimicrobial	
	Benzene, 1,2-dimethoxy-4-[[(4 methylphenyl)sulfonyl]methyl	Aromatic sulfur compound	Antimicrobial	
	Cholest-5-en-3-ol (3.beta.)-	Steroidal	Antibacterial	
	Ergost-5-en-3-ol, (3.beta.)-	Steroidal	Anti-microbial,	
	Stigmasta-5,22-dien-3-ol	Steroidal	Anti-oxidant, Anti-microbial, di-uretic	
	Stigmast-5-en-3-ol, (3.beta.)-	Steroidal	Anti-microbial, Anti-oxidant	
	Vitamin E		Anti-microbial, Anti-oxidant Hepato-protective,	
	alpha.-Tocopherol		Anti-microbial, Anti-oxidant	

Chapter 3:
OBJECTIVES

3. Objectives:

Based on the research survey and gaps in the current research following objectives were designed.

i) To study the effect the disinfectants presently in use.

ii) To study the natural plants and their parts with medicinal value.

iii) To study the compounds of plants with disinfectant property.

Chapter 4:
MATERIALS AND METHODS

4. Methods and methodology:

4.1 Over all Work flow chart for study

Text mining of different plants for their antimicrobial activity.

↓

Selection of plants according to their antimicrobial efficiency and availability.

↓

Extraction of extracts from different parts of selected plants.

↓

Standard Antimicrobial assays using different extracts.

↓

Standard Antimicrobial using combinations of different extracts.

4.2 Text mining for selection of plants with antimicrobial properties.

To examine the disinfectants currently in use, the drawbacks associated with it. And filling of the gaps with the natural plants with medicinal values was conductance on PubMed, google scholar, Collins' dictionary with the latest published papers till May 2020 independently. It was done using the key words, "anti-bacterial plants", "chemical disinfectants", "natural disinfectant".

4.3 To collect and identify plant materials

Plants were selected on the basis of their anti-microbial property along with their availability in northern plains.

4.4 Procedure (the procedure would be done in the lab if lockdown opened)

4.4.1. To prepare the extract using different plant parts of selected plants

4.4.2 To prepare the cultures from different sources, like one from LAF, one from open environment and another one from the lab environment and check the reduction rate due to natural disinfectants.

4.4.3 To analyze the phytochemicals, present in different plant parts. There could be number of compounds present on the extracted plant parts (alkaloids, terpenoids, flavonoids, etc.) and analyzed qualitatively using different procedures [Adetuyi, *et al*, 2001; Trease, *et al*, 1989; Sofowora, *et al*, 1982; Mujeeb, *et al*, 2014].

4.4.4 Antibacterial activity and % reduction calculation

Antibacterial count could be done using agar well-diffusion test.

% Reduction will be calculated using

$$\text{Reduction \%} = \frac{\text{Final count} - \text{Initial count}}{\text{Initial count}} \times 100$$

Chapter 5:
Results

5. Results:

In current state, the available chemical disinfectants do radiate some harmful effects like eye and skin irritation, may harm if inhaled, and other human health issues. Along with this, the continuous and increased uses of chemical disinfectants have developed resistance against the antimicrobials used to treat infections. Moreover, the concentrated commercial products can be hazardous, which are difficult to handle, store, and disposed by users. Thus using natural cleaning products may reduce the concerns related to human health and environment.

The most crucial part of present study was to identify the plants with antimicrobial properties, which could be further utilized as natural disinfectants. A comprehensive review of literature has been done successfully and active compounds of some plants along with their medicinal value have been highlighted. The plants selected were chosen on the basis that they show the antibacterial property and easy availability in the northern plains in India. These are commonly known as *Allium sativum* (garlic), *Syzygium aromaticum* (clove), *Juglans* (walnut), *Aegle marmelos* (bael), *Acacia* (acacia), and *Origanum vulgare* (oregano) and their active compounds carry antibacterial potential. The essential oils extracted from different plants like tea tree and clove is approved for antimicrobial properties. The oils from plants have shown activity to kill antibiotic-resistant strains of bacteria like MRSA. A diluted solution of essential oil is better linen spray. Similarly, a solution of vinegar and tea tree oil may use as multipurpose disinfectant/leaner. Thus, extracts may be prepared with these plants can be used by mankind for the preparation of natural disinfectants. During present study, the labs could not be accessed because of Covid-19 thus *in-vitro* experiments could not be performed. From the literature survey, it was elucidated that *Acacia* is found to have different active compounds and found to impart antimicrobial as well as anti-biofilm activity. Similarly, *Allium sativum* is found to have Allicin, DADS, DAS, DATS, E/Z- ajoene, alliin, SAC as active compounds and can be used as antimicrobial, to lower the blood pressure. *Juglans regia* are also high medicinal valued plant with its kernel part being rich in p-hydroxybenzoic acid, vanillic acid, gallic acid, quinic acid, epicatechin gallate, rutin, naringin, quercetin-3-D-glucoside, syringic acid, while the dried bark of *Juglans regia* is known to be dandasa. It shows anti-cancerous, anti-biofilm and antibacterial properties.

Green tea also has catechins, flavonoids as active compound such as epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin (EC), epicatechin gallate (ECG), which are found to be imparting antibacterial as well as anti-biofilm effect. *Origanum vulgare* has been reported with rosmarinic acid, caffeic acid, protocatechuic acid, coumaric acid and quercetin; along with thymol and carvacrol. These compounds help in imparting anti-oxidant property. *Aegle marmelos* have been also reported with a list of active compounds which shows antibacterial, anti-microbial, antifungal properties.

In conclusion, after screening natural compound with antimicrobial potential, the present study suggests indigenous or combined plant extracts may have potential to be utilized as natural disinfectants.

Chapter 6:
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