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Role of Polyphenols from Plants on Enterotoxin Producers

Aarsh Sane[1], Hitesh Gurav[2]
[1]Undergraduation student in department of Biotechnology at S.S & L.S Patkar Varde College Of Science, Mumbai, India.
[2]Assistant Professor in department of Biotechnology at S.S & L.S Patkar Varde College Of Science, Mumbai, India.

Abstract: In this study we are going to see the role of different polyphenols extracted from plants and their anti-microbial properties especially towards enterotoxins producing organism. In this article we have targeted four major disease causing microbial agent viz. *Staphylococcus aureus, Vibrio cholerae, Clostridium & Escherichia coli*. It was investigated that tea catechins, dihydroisosteviol, RG-tannins, apple phenols Red chili, Sweet fennel, White pepper, Red bayberry, Thymol, Carvacrol are some of the plant compounds having anti bacterial properties against *Vibrio cholrae* . They inhibit the toxins by modulating transmembrane regulators and inhibition of ADP-ribosyltransferase activity. Similar study is done on other three organisms. As we have seen many microbes have developed multidrug resistance and thus it is important to study about different plant phenols having anti-microbial activity. Majority of the plant compounds were extracted using LLE method. The methanol extracts were found to inhibit the growth of most of the microorganisms as compared to the aqueous extracts. These findings suggest that the methanol extracts of the plants tested contain compounds with antimicrobial properties. These exhibited properties propose that such plant extracts have a great scope to be used as natural preservatives in the food and pharmaceutical industries.

Keywords: Polyphenols, Enterotoxins, *Staphylococcus aureus* enterotoxins A, Grape pomace, Tanins, Clostridial toxin A (TcdA), Oleuropein.

I. INTRODUCTION

Microbial illness especially bacterial diseases are a leading cause of mortality in humans. Bacteria synthesis different types of toxins which can be grouped into three types viz. 1. Endotoxins: which is a lipopolysaccharide formed in the outer membrane of gram negative bacteria, 2.Exotoxin: a protein secreted by gram positive bacteria, 3. Enterotoxins: A type of exotoxin affecting human's digestive system, entering into stomach via contaminated food causing cramps nausea & vomiting. They can be encoded chromosomally/ in plasmids. They are heat stable and secreted outside the bacterial cell. These are cytotoxic and hence kill epithelial cells altering apical membrane permeability by forming pores and leaking the chloride ions into the lumen thus increasing the sodium ion and water content into lumen causing diarrhea.

There are three types of Enterotoxins 1. Exemplified by diphtheria toxin, they form pores/ holes viz. aerolysin synthesized by *aeromonas hydrophila.* 2. Super antigen toxin: they over stimulate the immune response with respect to T-cells. Viz. *Streptococcus aureus*& *Streptococcus pyogenes*. 3. A-B Toxin: consist of two or more toxin subunit. The subunit A binds and form channels by binding to the receptor for subunit B to enter in. All the toxins play a major role in virulence and pathogenic properties of the microbes and thus act as a target for developing therapeutic inventions. Currently it has been noticed that antimicrobial resistance has been developed due to continuous exposure of certain drugs and the organism have become more pathogenic or virulent. This has given a

push to natural medicines. Among such therapeutic preparations are plant-derived phytomedicines cosmaceuticals, and nutraceuticals.

Many plants have important multifunctional properties derived from their specific bioactive components. Biologically active components of plants are mostly secondary metabolites, viz; terpenoids, glycosides, phenolics and alkaloids, present as alcohols, aldehydes, ketones, esters, ethers, and lactones [7]. They act by iron deprivation and interactions with vital proteins example enzyme. Tannins are polyphenolic secondary metabolites, classified in two groups: hydrolysable tannins which are present in plants as gallotannins or ellagitannins and condensed tannins (CT) [7]. Gallotannins from mango kernel inhibited the growth of *Bacillus subtilis* and other gastrointestinal microbes such as *S. aureus* and *E. coli.* The inhibitory effects may be attributed to their iron-complexing properties and ability to interact with proteins and inhibit enzyme activity [8]. The phenolic compounds suppress the bacterial biofilm formation by the inhibition of different regulatory mechanisms without affecting growth, can block the quorum sensing, reduce bacterial motility, superficial adhesion, and inhibit expression of bacterial factors related to pathogenic behavior [8].

It is now possible to increase the antimicrobial activity of plant extracts by certain processes. Antimicrobial effect against different types of bacteria may depend on the cocktail of enzymes used for the treatment as well. Example: Flaxseeds & Pumpkin extracts, treatment using a mixed cocktail of immobilized Aspergillus oryzae –amylase and Aspergillus niger -glycosidase and -glucanase enzymes resulted elevated microbial activity against pathogenic microbes [8]. Red grape pomase subjected to cellulose-assisted extraction efficiently inhibited the growth of *E.coli & S.aureus.* Carvacrol, a plant compound having antimicrobial activity against Botulinum neurotoxin, Tcd A, Tcd B produced by gram positive bacteria [17].

There are many techniques and procedure for extracting phenols. Commonly used is LLE (liquid-liquid extraction) including soxhlet method, maceration & hydro distillation method. This is based on types and polarity of the solvent and their ratios, time & temperature of extraction, moreover chemical composition and physical characteristics of sample. Ultrasound-assisted extraction (UAE), Superficial fluid extraction (SFE) and microwave assisted extraction (MAE) are some other techniques. Similarly ELISA (enzyme linked immune sorbent assay), Chemiluminescence immunoassay (CLIA), reversed passive latex agglutination test are used to detect enterotoxins. Elek's test is specifically used for *Corynebacterium diptheriae.*

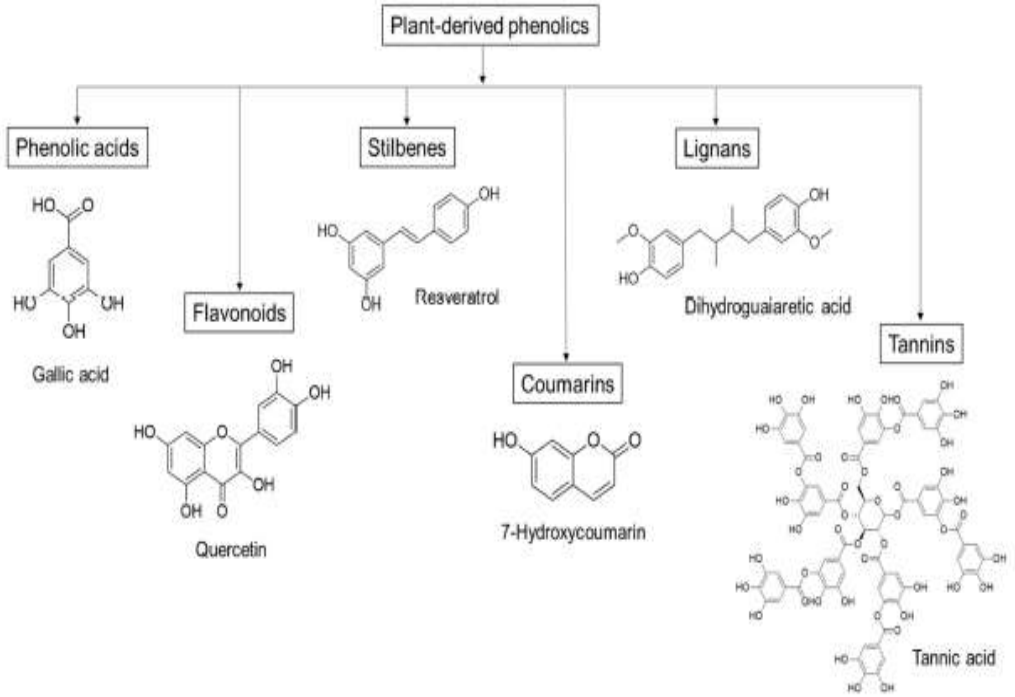

Figure1: Plant derived phenolics [19]

II. EFFECT OF PLANT COMPOUNDS ON GRAM POSITIVE BACTERIAL ENTEROTOXINS

2.1 *Staphylococcus auereus:*

The anti-enterotoxins activities are intensively tested for food borne pathogens especially S.aureus. It produces enterotoxins, exfoliative toxins and toxic shock syndrome toxin-1 that exert stress on host immune system and also on vital organs. Exotoxin including hemolysins (alpha, beta, gamma, and delta) nucleases, proteases and collagenase that mediate host tissue digestion for bacterial nourishment and growth are synthesized. Different plant compounds with sub-inhibitory concentrations were found to be useful against these enterotoxins [8] [17]. Essential oils from clove and cinnamon reduce enterotoxins A and B production, alpha hemolysin in vitro [1]. It was found that euglenol reduce the expression of toxin producing genes including sea, seb, tst, and hla coding for various stages of toxin production in S. aureus. Similar effect were seen in some other plant compounds including Origanum vulgare or oregano , Zataria multiflora Boiss, a spice plant found in mid-west Asia and 4-hydroxytyrosol[12]. *S.aureus* produces group of 11 enterotoxins (SE) of which staphylococcal enterotoxin A (SEA) is the most common. It is noted that apple polyphenols have inhibitory activity towards SEA. Apples contain many phenolic compounds (i.e., chlorogenic acid, (+)-catechin, epicatechin, rutin, phloridzin, and oligomeric procyanidins (PC).

Tannins, extracted from raw apple juice, contained polymerized catechins including pentadecamers. It was observed that highly polymerized PC comprised in AP interacts with the A-6 region. It was reported that tannins have binding affinity to the lipase present in pancreatin. Proanthocyanidins bind to the amylase in pancreatin & the interaction between SEA and AP was weakened due to the binding of pancreatin enzyme. SEAs are superantigenic toxins which form the bridge between antigen presenting cells and T cells. The cross-linkage formation induces T-cell proliferation and produces T-cell-activating cytokines such as IFN-gamma. It was seen that apple juice inhibited SEA induced T-cell proliferation by blocking the connection between AP and T-cells. Also the oligomeric procyanidines interfered with glycolysis and inhibited the functions of activated T-cells. It was suggested that oligomeric PCs have an influence on the interaction of ligands and their receptors on the cell surface, including the surface of CD4+ T cells [15]. Some polyphenols extracted from walnut including Lara, Franquette, Mellanaise, Mayette, Marbot and Parisienne also showed anti-enterotoxin properties against *S.aureus* [13]. Walnut leaves are also a good source of healthy phenolic compounds suggesting that it could be useful in the prevention of diseases in which free radicals are implicated. Portulaca oleracea L. used as a Chinese herbal medicine contains flavanoids (POFE) which was found to be sensitive against *S.aureus*.it was noted that POFE-treated cells displayed various apoptotic markers, such as reactive oxygen species (ROS) accumulation, membrane depolarisation, phosphatidylserine exposure, DNA fragmentation, and caspase-like protein, in a dose-dependently manner which caused cell death. Induction of ROS synthesis leads to the formation of highly reactive radicals that are lethal to cells. Excess generation of ROS can attack bacterial cell membrane lipids and lead to a breakdown of bacterial cell membrane function [22].. Moringa oleifera leaves extract also showed growth inhibition for S.aureus [9]. González-Fandos et al.(1994) demonstrated the influence of garlic on enterotoxin production by *Staphylococcus aureus*[1]. Assays prooved that sub inhibitory concentration of Helichrysum italicum extract also inhibited the enzymatic activity and production of enterotoxins. Essential oils (food grade) derived from clove (Eugenia caryophyllata), thyme (Thymus vulgaris) and cinnamon (Cinnamomum zeylanicum) also showed sub-inhibitory effect againt enterotoxins of *S.aureus.*

TABLE 1. Unique features of some Staphylococcal enterotoxins [1]

Staphylococcal Enterotoxin	Feature	Binding to Class II MHC
SEA	Most common toxin associated with staphylococcal food poisoning	Alpha and beta chains [12]
SEB	Studied as a biological weapon	Alpha chain [13]
SEC	Commonly isolated from animals [14]	Outside the binding groove on the flanking helix from the α chain [15]
SED	Food poisoning [16]	Alpha and Beta chains [17]
SEE	Food poisoning [9]	Beta chain [18]
SEF	Associated with toxic shock syndrome [8]	Binds to alpha and beta chains [19]
SEG	Minor role in food poisoning [10]	SEB-like interaction with a chain [20]
SEH	Food poisoning [10,11]	Alpha chain [21]
SEI	Minor role in food poisoning [10]	Beta chain [22]

2.2 *Vibrio cholera:*

Cholera is an infectious disease that causes severe watery diarrhea, and is responsible for dehydration and even death if untreated. It is caused by *Vibrio cholerae* present in contaminated food and water. It produces AB5 kind of toxin called as CT toxin which is responsible for the bulk infections. The pathogen attaches to intestinal epithelial cells using toxin co-regulated pilus (tcpA) .After which the pathogen secretes cholera toxin that is internalized by intestinal epithelial cells, followed by processing within the endoplasmic reticulum. Dissociation of A and B subunit leads to ADP-ribosylation of the alpha subunits of the heteromeric Gs proteins, leading to the activation of the adenylate cyclase pathway causing an increase in chloride ion secretion and blocking of sodium influx resulting in an increased solute concentration within the lumen resulting in severe diarrhea[1].

Research based on use of plant derived polyphenols specially extracted from tea leaves and grape extract has shown some positive and beneficial results against this CT toxin. Using a novel cell-based assay it had been identified that grape seed and grape skin extracts act as potent inhibitors of CT intoxication of cultured cells and intestinal loops [4].. Anti-CT properties of grape extract included (i) stripping pre-bound toxin from the cell surface; (ii) blocking the unfolding of the isolated A1 chain; (iii) disrupting the ER-to-cytosol export of CTA1 and (iv) Inhibiting the catalytic activity of CTA1. Yet the extract failed to affect toxin transport from the cell surface to the ER or the dissociation of CTA1 from its holotoxin [4].It was revealed that the two compounds that prevent toxin binding at the cell surface epigallocatechingallate (EGCG) and procyanidin B2 (PB2) also strip pre-bound CT from the plasma membrane and are predicted to engage the GM1 binding site of CTB by docking simulations. Grape extracts do not prevent downhill CT transport from the plasma membrane to the Endoplasmic reticulum or the ER-localized release of CTA1 from the rest of the toxin, but they do block the thermal unfolding and export of CTA1 from ER to cytosol [4].

Yamasaki and coworkers investigated effect of spices such as red chili, sweet fennel, and white pepper on cholera toxin production process. It was found out that Capsaicin was the major component among the tested spices which reduced the expression of critical virulence genes including ctxA, tcpA, and toxT that code for toxin production in *V. cholera*e. Capsaicin also enhanced the expression of hns gene that negatively regulates toxin production genes [1].

TABLE 2: Plant compounds against some microbial toxins [1].

Microbe	Toxin(s)	Plant Compounds with Anti-Toxin Activity	Potential Mechanism of Action/Target Site
Gram negative bacteria			
Vibrio cholerae	Cholera toxin	Tea catechins, Dihydroisosteviol	Modulation of transmembrane regulators
		RG-tannin, apple phenols	Inhibition of ADP-ribosyltransferase activity
		Red chilli, sweet fennel, white pepper Red bayberry, thymol, carvacrol, eugenol	Modulation of toxin production genes *ctxA*, *tcpA*, *toxT*
Toxin producing *E. coli*	ETEC toxin	Extracts from *Galla Chinensis* and *Berberis aristata*, leanolic acid, ursolic acid, and betulinic acid, essential oil from *Cymbopogon martini*, *C. winterianus* and *Psidium guajava*	Inhibiting intestinal secretion of ETEC enterotoxins Blocking the binding of heat labile enterotoxin to GM1 Reducing toxin binding and toxin mediated cellular pathology
	Verotoxin	Extracts from *Limonium californicum (Boiss.) A. Heller*, *Cupressus lustianica Miller*, *Salvia urica Epling* and *Jusiaea peruviana L.*, eugenol, catechin, epigallocatechin, cinnamon bark oil, cinnamaldehyde, *Curtisia dentata* extract *carvacrol*, *thymol*, *beta-resorcylic acid*, grape seed and pomace extracts	Decrease in toxin production Reducing the transcription of *stx1* and *stx2* genes Reducing the expression of globotriaosylceramide (Gb3) receptor by mimicking toxin receptors

2.3 Enterotoxigenic *Escherichia coli* (ETEC):

Escherichia coli (*E.coli)* is a Gram-negative organism, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded animal. It is one of the normal microflora present in human intestine. The major pathogenic *E.coli* includes enterotoxigenic (ETEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), diffusely adherent (DAEC) and enteroaggregative E.coli (EAEC) [1]. They cause illness in humans including gastroenteritis, septicemia, and meningitis. E. coli O157:H7 (EHEC) is responsible for approximately 73,000 foodborne diseases. In the case of EHEC, verotoxins are the major virulence factors that cause intestinal and renal epithelial damage leading to hemorrhagic colitis and hemolytic uremic syndrome, respectively [1].

Enteroaggregative E. coli attaches to intestinal epithelium and releases cytotoxins and enterotoxins in the extracellular lumen causing crypt orifice dilation, rounding and extrusion of enterocytes. Natural extracts from Galla chinensis and Berberis aristata have been used in traditional Chinese medicine for the treatment of diarrhea [2] [18]. Similar anti-toxic activity with other plant derived compounds namely, oleanolic acid, ursolic acid, and betulinic acid is also seen. Carragenan, essential oil from Cymbopogon martini and C.winterianus, Psidium guajava, thiols and disulfide compounds has been reported. Sakagami and coworkers investigated the effect of extracts from plant species namely, Limonium californicum and found out that it had the highest antimicrobial property against E.coli bacteria.

2.4 Clostridium:

Clostridium difficile is a nosocomial gram positive pathogen that causes severe gastrointestinal disease and is responsible for stimulation of inflammation and disease. It *produces* two major toxins viz. clostridial toxin A (TcdA) and clostridial toxin B (TcdB). They cause damage to mucosal pithelium in gut. Vancomycin, fidaxomicin, and metronidazole are antibiotics currently used for the treatment of *C.difficile* infections. More difficult to treat *C. difficile* hyper virulent ribotype 027/NAP1/BI strain with fidaxomicin and vancomycin than other strains.

Pomegranate fruit is found to be useful both nutritionally and as a medicine agent. It is rich in polyphenols. The major ellagitannin (ET) called punicalagin (2, 3-hexahydroxy-diphenoyl-4, 6-gallagylglucose) is the abundant type of polyphenol present in the pomegranate juice. The pomegranate juice contains polyphenols:- 1561 mg/L of punicalagins, 387 mg/L of anthocyanins, 121 mg/L of EAs, and 417 mg/L of other hydrolyzable tannins[16].Pomegranate juice containing the concentration of punicalagin decreased the expression of TcdB. POMx is a pomegranate extract (husks, seeds, and peel remaining after juice production), and pomegranate juice stimulated the growth of bifidobacteria and lactobacilli and inhibited the growth of *B. fragilis* group, Clostridia, and Enterobacteriaceae. The probiotic bacterial species such as lactobacilli and bifidobacteria inhibits the growth of pathogenic *C. difficle.*

Necrotic enteritis is a worldwide extended disease caused by *Clostridium perfringen*. It is an ubiquitos gram positive organism. *C.perfringes* is a spore forming, toxigenic, anaerobic bacterium, generally classified according to the production of five major toxins. Clinical signs of this disease are decreased appetite, diarrhea, weight loss, and several nonspecific signs that can be gross lesions affecting small intestine, liver and ileum jejunum [5]. They are mainly multi drug resistant and hence plant polyphenols are being used to treat this disease. Many plants have beneficial multifunctional antibacterial properties derived from their bioactive components. Biologically active components of plants are mostly secondary metabolites viz. terpenoids, phenolics, glycosides, and alkaloids are present as alcohols, aldehydes, ketones, esters, ethers, and lactones. Tannins act by iron deprivation and interactions with vital proteins such as enzyme; Cryptolepine, the main indoloquinoline alkaloid, is a DNA intercalator and inhibitor of topoisomerase; Saponins form complexes with sterols from the membrane of microorganisms and causes damage and consequent collapse of the cells. The antimicrobial activity has been linked to their biochemical properties including metabolism inhibition by enzyme complexation and iron deprivation. Addition of a chestnut tannin extract helps reducing the counts of CP and macroscopic gut lesions in broiler chickens challenged with coccidia and *C.perfringes*. There are numerous reports about the antibacterial effects of essential oils like Origanum vulgare, Piper nigrum, Syzygium aromaticum, and Thymus vulgaris, and their components, thymol, carvacrol, and eugenol, against *Clostridium* species including CP.

TABLE 3: Most common food borne pathogenic bacteria, their produced toxins and diseases caused by them [19]

Foodborne Pathogen Bacteria	Toxin Production	Type of Disease	Main Food Sources of Infection
Bacillus cereus	Emetic toxin, diarrheal toxin	Emetic syndrome, diarrhea	Rice, pasta, noodles, pastry
Campylobacter coli, Campylobacter jejuni	Cytolethal distending toxin	Campylobacteriosis	Poultry products, unpasteurized milk
Clostridium botulinum	Botulinum toxin	Botulism	Improperly processed canned foods
Escherichia coli O157:H7	Shiga-toxin	Hemorrhagic colitis	Ground meats, raw or under-pasteurized milk, sprouts
Listeria monocytogenes	Listeriolysin O	Listeriosis	Soft cheeses from unpasteurized milk, ready-to-eat products
Salmonella Typhi, *Salmonella* Typhimurium, *Salmonella* Enteritidis	Enterotoxin	Typhoid fever, salmonellosis (gastroenteritis)	Any type of food: meat, poultry, fish, milk, eggs, vegetables, water
Staphylococcus aureus	Heat stable enterotoxins	Gastrointestinal symptoms	Meat, dairy products, salads
Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus	Cholera toxin	Cholera, gastroenteritis	Raw/undercooked shellfish, meat, contaminated water

III. PLANT PHENOLIC EXTRACT AS ANTI-MICROBIAL AGENTS.

3.1 Oleuropein in Olive and its Anti-microbial Effects: The olive, known by the botanical name Olea europaea, "European olive", is a species of small tree within the family Oleaceae, found in the Mediterranean Basin. It has been revealed that the olive oil constitutes oleic acid, phenolic constituents and squalene. The hydroxytyrosol and oleuropein are main phenolic compounds. Oleuropein have important medical uses like antioxidant, anti-inflammatory, anti-cancer activities, antimicrobial activity,antiviral activity, hypolipidemic and hypoglycemic effect. The biosynthesis of oleuropein in Olives proceeds via mevalonic acid pathway, resulting in the formation of oleosides. Oleuropein is found in abundant quantity in the early stages that are in young fruits, it has capability to reach 14% of dry matter. The fruit of Olea europaea tends to accumulate only glucosylated derivatives of oleuropein. On the opposite hand, dihydroxytyrosol and non-glucosylated secoiridoids derived from oleuropein have been found in the leaves. Oleuropein has been shown to have strong antimicrobial activity against both Gram-negative and Gram-positive bacteria & mycoplasma [10].Different authors have used biophysical assays to check the interaction between oleuropein and membrane lipids however; the exact mechanism of the antimicrobial activity of oleuropein is still not completely established. Oleuropein and its hydrolysis products are able to inhibit the development and production of enterotoxin B produced by *Staphylococcus aureus*. Oleuropein and other phenolic compounds including p-hydroxybenzoic, vanillic and p-coumaric acids completely inhibit the growth of Klebsiella pneumoniae, *Escherichia coli* and *B. cereus.*

3.2 Naphtoquinones from Walnut (Genus Juglans; Family Juglandaceae): The leaves of walnut (Lara, Franquette, Mayette, Mellanaise and Parisienne) grown in Portugal, were analysed in what concerns phenolic compounds and antimicrobial and antioxidant properties. The antimicrobial capacity was screened against Gram positive organisms viz. (*Bacillus cereus, S. aureus*) and Gram negative bacteria (*Pseudomonas aeruginosa, Escherichia coli, K.pneumoniae*) and fungi (Candida albicans, Cryptococcus neoformans). Lara walnut leaves were introduced to antibacterial assays using 18 clinical isolates of Sp. Antioxidant activity was checked by the Staphylococcus reducing power assay, the scavenging effect on DPPH (2, 2-diphenyl-1-picrylhydrazyl) radicals and b-carotene linoleate model system. Naphtoquinones and flavonoids are major phenolic compounds. Extracts presented similar antimicrobial capacity, inhibiting only Gram+ve bacteria and in the order B. cereus>> S. aureus>> subtilis [13]. The black walnut, Junglas nigra, belonging to eastern North America contains phenolic compounds that exhibit antioxidant and antimicrobial properties. Walnuts are a rich source of antioxidants, natural compounds

demonstrated to have numerous health benefits and antimicrobial property.viz. Proanthocyanidins, which are present in high amounts in nuts, are known to inhibit staphylococcal toxins [21].

TABLE 4: Antimicrobial activity of leaf extract of different walnut cultivars [21].

Cultivar	MIC (mg/mL)							
	B. cereus	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*	*K. peumoniae*	*C. albicans*	*C. neoformans*
Lara	0.1 (+ + + +)	10 (+ + + +)	0.1 (+ + + +)	100 (–)	100 (–)	100 (–)	100 (–)	100 (–)
Franquette	0.1 (+ +)	10 (+ + +)	0.1 (+ +)	100 (–)	100 (–)	100 (–)	100 (–)	100 (–)
Mellanaise	0.1 (+ +)	10 (+ + +)	0.1 (+ +)	100 (–)	100 (–)	100 (–)	100 (–)	100 (–)
Mayette	0.1 (+ +)	10 (+ + + +)	1 (+ + + +)	100 (–)	100 (–)	100 (–)	100 (–)	100 (–)
Parisienne	0.1 (+ +)	10 (+ +)	1 (+ + + +)	100 (–)	100 (–)	100 (–)	100 (–)	100 (–)
Marbot	0.1 (+ + + +)	10 (+ +)	1 (+ + + +)	100 (–)	100 (–)	100 (–)	100 (–)	100 (–)

No antimicrobial activity (–), inhibition zone <1 mm. Slight antimicrobial activity (+), inhibition zone 2–3 mm. Moderate antimicrobial activity (+ +), inhibition zone 4–5 mm. High antimicrobial activity (+ + +), inhibition zone 6–9 mm. Strong antimicrobial activity (+ + + +), inhibition zone >9 mm. Standard deviation ±0.5 mm.

3.3 Extracts from Artemisia & Saponaria officinalis: Wormwood is typically used as an extract or tea. Its oil is obtained from the stems and leaves of the plant, whereas an extract or tincture may use the entire plant. These formulations lack calories, vitamins, or minerals but contain numerous plant compounds having antimicrobial, anti-inflammatory & pharmacological properties. The *Artemisia absinthum* extract also showed antibacterial activity against majority of tested microorganisms (6-19 mm inhibition zone), apart from *Alcaligenes feacalis* and *Aspergillus niger* [17]. In addition, a positive correlation (R = 0.819) between the total phenolic content and antioxidant activity in the plant samples were found out. It was noted that the tarragon essential oil has antimicrobial properties. The oil showed different levels of inhibitory effect on growth of gram positive bacteria which are responsible for the majority of enterotoxins diseases. *E.coli* showed more sensitivity towards oil as compared to *S.aureus* [11].

Soapwort (Saponaria officinalis) is a perennial plant in the Caryophyllaceae family that is used to create a natural soap/cleanser. The leaves/roots/stems can be boiled to make **soapwort** cleansers. The methanol extracts of the plants samples are found to be more effective on microorganisms in comparison to the aqueous extracts. The methanolic extract of *Artemisia santonicum exhibit* antibacterial activity against majority microorganisms. Memnune sengul et al. noted that the inhibition effects of the methanol extracts of *Artemisia absinthum* and *Artemisia santonicum* against some microorganisms such as *Bacillus subtilis, Salmonella typmiruim, Saccharomyces cereviciae, Bacillus cereus, Streptococcus thermophilus, Providenci alcaliaciens* and *Pseudomonas putida values* were higher than that of the positive controls [17].

IV. CONCLUSION AND FUTURE SCOPE

Plants have diverse polyphenols having a broad spectrum of antimicrobial properties. Over the past few decades there is an evolution seen in use of this polyphenols and other important plant compound against majority of the toxin producing organism. Moreover, in-depth studies are required to properly characterize the therapeutic/chemical effects of plant compounds in the host for developing reliable and effective drug. In order to support the use of natural plant products and to become acceptable by the mainstream industry market, different research groups provided solid scientific evidence addressing the issue of inconsistency across many studies in literature. There is no plant with zero anti-microbial properties. This defense system of plants can be a great advantage and opportunity to the human diversity. Polyphenols derived from plant could be the great future against multi-drug resistant microorganism.

REFERENCES

1. Aggarwal, S., Jena, S., Panda, S., Sharma, S., Dhawan, B., Nath, G., Singh, N. P., Nayak, K. C., & Singh, D. V. (2019). Antibiotic Susceptibility, Virulence Pattern, and Typing of *Staphylococcus aureus* Strains Isolated From Variety of Infections in India. *Frontiers in microbiology*, *10*, 2763. https://doi.org/10.3389/fmicb.2019.02763
2. Baskaran, X. R., Geo Vigila, A. V., Zhang, S. Z., Feng, S. X., & Liao, W. B. (2018). A review of the use of pteridophytes for treating human ailments. *Journal of Zhejiang University. Science. B*, *19*(2), 85–119. https://doi.org/10.1631/jzus.B1600344
3. Bhandari S, Khadayat K, Poudel S, et al. Phytochemical analysis of medicinal plants of Nepal and their antibacterial and antibiofilm activities against uropathogenic Escherichia coli. BMC Complement Med Ther. 2021; 21:116. Published 2021 Apr 9. doi:10.1186/s12906-021-03293-3.
4. Cherubin, P., Garcia, M. C., Curtis, D., Britt, C. B., Craft, J. W., Jr, Burress, H., Berndt, C., Reddy, S., Guyette, J., Zheng, T., Huo, Q., Quiñones, B., Briggs, J. M., & Teter, K. (2016). Inhibition of Cholera Toxin and Other AB Toxins by Polyphenolic Compounds. *PloS one*, *11*(11), e0166477. https://doi.org/10.1371/journal.pone.0166477
5. Diaz Carrasco, J. M., Redondo, L. M., Redondo, E. A., Dominguez, J. E., Chacana, A. P., & Fernandez Miyakawa, M. E. (2016). Use of Plant Extracts as an Effective Manner to Control *Clostridium perfringens* Induced Necrotic Enteritis in Poultry. *BioMed research international*, *2016*, 3278359. https://doi.org/10.1155/2016/3278359
6. Erian, Nazmy & Hamed, Hassan & Nihad, Dr & Elhalwagi, Abeer & Elhamid, Ebtihal & Farid, Mohamed. (2016). Biochemical Studies on Moringa Oleifera Leaves Extract.
7. Juneja, V. K., Bari, M. L., Inatsu, Y., Kawamoto, S., & Friedman, M. (2007). Control of Clostridium perfringens spores by green tea leaf extracts during cooling of cooked ground beef, chicken, and pork. *Journal of food protection*, *70*(6), 1429–1433. https://doi.org/10.4315/0362-028x-70.6.1429
8. Mishra* et al., 5.(6): June, 2016. INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGYEFFECT OF COFFEE ON MICRO ORGANISM
9. Nazzaro, F., Fratianni, F., & Coppola, R. (2013). Quorum sensing and phytochemicals. *International journal of molecular sciences*, *14*(6), 12607–12619. https://doi.org/10.3390/ijms140612607
10. Omar S. H. (2010). Oleuropein in olive and its pharmacological effects. *Scientia pharmaceutica*, *78*(2), 133–154. https://doi.org/10.3797/scipharm.0912-18
11. Osanloo, M., Amani, A., Sereshti, H., Abai, M. R., Esmaeili, F., & Sedaghat, M. M. (2017). Preparation and optimization nanoemulsion of Tarragon (Artemisia dracunculus) essential oil as effective herbal larvicide against Anopheles stephensi. *Industrial crops and products*, *109*, 214-219
12. Parsaeimehr, M., Basti, A. A., Radmehr, B., Misaghi, A., Abbasifar, A., Karim, G., Rokni, N., Motlagh, M. S., Gandomi, H., Noori, N., & Khanjari, A. (2010). Effect of Zataria multiflora Boiss. essential oil, nisin, and their combination on the production of enterotoxin C and alpha-hemolysin by Staphylococcus aureus. *Foodborne pathogens and disease*, *7*(3), 299–305. https://doi.org/10.1089/fpd.2009.0416
13. Pereira, José & Oliveira, Ivo & Sousa, Anabela & Valentão, Patrícia & Andrade, Paula & Ferreira, Isabel & Ferreres, Federico & Bento, Albino & Seabra, Rosa & Estevinho, Leticia. (2007). Walnut (Juglans regia L.) leaves: Phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association. 45. 2287-95. 10.1016/j.fct.2007.06.004
14. Pinchuk, I. V., Beswick, E. J., & Reyes, V. E. (2010). Staphylococcal enterotoxins. *Toxins*, *2*(8), 2177–2197. https://doi.org/10.3390/toxins2082177
15. Shimamura, Y., Hirai, C., Sugiyama, Y., Utsumi, M., Yanagida, A., Murata, M., Ohashi, N., & Masuda, S. (2017). Interaction between Various Apple Procyanidin and Staphylococcal Enterotoxin A and Their Inhibitory Effects on Toxin Activity. *Toxins*, *9*(8), 243. https://doi.org/10.3390/toxins9080243
16. Sukumar, M. R., & König, B. (2018). Pomegranate extract specifically inhibits *Clostridium difficile* growth and toxin production without disturbing the beneficial bacteria in vitro. *Infection and drug resistance*, *11*, 2357–2362. https://doi.org/10.2147/IDR.S163484
17. Sengul, M., Ercisli, S., Yildiz, H., Gungor, N., Kavaz, A., & Cetin, B. (2011). Antioxidant, Antimicrobial Activity and Total Phenolic Content within the Aerial Parts of Artemisia absinthum, Artemisia santonicum and Saponaria officinalis. *Iranian journal of pharmaceutical research : IJPR*, *10*(1), 49–56.
18. Sack, R. B., & Froehlich, J. L. (1982). Berberine inhibits intestinal secretory response of Vibrio cholerae and Escherichia coli enterotoxins. *Infection and immunity*, *35*(2), 471–475. https://doi.org/10.1128/IAI.35.2.471-475.1982
19. Takó, M., Kerekes, E. B., Zambrano, C., Kotogán, A., Papp, T., Krisch, J., & Vágvölgyi, C. (2020). Plant Phenolics and Phenolic-Enriched Extracts as Antimicrobial Agents against Food-Contaminating Microorganisms. *Antioxidants (Basel, Switzerland)*, *9*(2), 165. https://doi.org/10.3390/antiox9020165

20. Upadhyay, A., Mooyottu, S., Yin, H., Nair, M. S., Bhattaram, V., & Venkitanarayanan, K. (2015). Inhibiting Microbial Toxins Using Plant-Derived Compounds and Plant Extracts. *Medicines (Basel, Switzerland)*, *2*(3), 186–211. https://doi.org/10.3390/medicines2030186
21. Wenzel, J., Storer Samaniego, C., Wang, L., Burrows, L., Tucker, E., Dwarshuis, N., Ammerman, M., & Zand, A. (2016). Antioxidant potential of *Juglans nigra*, black walnut, husks extracted using supercritical carbon dioxide with an ethanol modifier. *Food science & nutrition*, *5*(2), 223–232. https://doi.org/10.1002/fsn3.385
22. Yong-Kai Du, Jing Liu, Xiu-Mei Li, Fang-Fang Pan, Zhi-Guo Wen, Tong- Cun Zhang & Pei-Long Yang (2017) Flavonoids extract from Portulaca.oleracea L.induceStaphylococcus.aureus death by apoptosis-like pathway, International Journal of Food Properties, 20:sup1, S534-S542, DOI: 10.1080/10942912.2017.1300812. https://doi.org/10.1080/10942912.2017.1300812