THE ANTIBACTERIAL EFFECTS OF ETHANOL AND WATER EXTRACTS OF. *ALLIUM CEPA.* L: A Review

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ABSTRACT: *This review article compiles and summarizes extensive literature evidence through the researches reported on the antibacterial activity of Allium cepa L with ethanol and water extract. The literature collected is based on the searches from, PubMed, ScienceDirect and Google scholar search engines covering a period of 2010 to 2020BC. In accordance with the articles evaluated for the antibacterial activity of ethanol and water extract of Allium cepa. L, zone of inhibition (ZOI), minimal bactericidal concentration (MBC), minimal inhibitory concentration (MIC), and concentration of the ethanol extract has a high inhibitory effect as compared to water extract against some bacteria, such as Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Proteus mirabilis and Salmonella spp. The most discussed bacteria in all the articles reviewed were Staphylococcus aureus and Escherichia coli. The Allium cepa. L, extract is used with different ethanol concentrations. The highest concentration is 50% for S. Aureus. The greatest inhibition zone observed is with Staphylococcus aureus and Escherichia coli with a diameter of 23 mm. Bacillus cereus was inactivated by the greatest concentration (125 mg/ml). MBC observations of all articles were carried out on four types of bacteria: Listeria monocytogenes (500 µg / ml), in red onion (Pseudomonas aeruginosa,(6.25 % v/v) Staphylococus aureus (-) and Escherichia coli (-)) in white onion Pseudomonas aeruginosa,(6.25 % v/v) Staphylococus aureus (-) and Escherichia coli (50 % v/v). The antibacterial activity of the ethanol extract was better than the water extract.*

Keywords: *Antibacterial, Allium cepa L., Ethanol Extract, Water Extract, Zone of Inhibition (ZOI), Minimum Inhibition Concentration (MIC), Minimum Bactericidal Concentration (MBC)*

INTRODUCTION

Antibacterial substances can interfere with growth or even kill the bacteria by disrupting their harmful microbial metabolism [1]. Antibacterial activity is used to prevent infectious diseases as well as to treat some diseases. Bacterial infections have a large impact on public health. All of the human organs are susceptible to bacterial infections. For example, *Neisseria meningitidis* normally infects the meninges (covering) of the central nervous system, causing meningitis, and can also infect the lungs, causing pneumonia [2].

Active compounds in plants have antibacterial properties, which includes the secondary metabolite compounds. Secondary metabolite compounds are the alkaloids, flavonoids, saponin, tannin, steroids and triterpenoids [3]. *Allium cepa* L is a biennial glabrous herb, usually growing as an annual from seeds or bulbs, up to 100 cm tall (Figure. 1). *Allium cepa* L. has essential oils rich in phenolic compounds. Some of these phenolic compounds in onion include phenolic acids derived from benzoic acid or cinnamic acid, such as protocatechuic acid, gallic acid, p-hydroxybenzoic acid as well as flavonoids, such as kaempferol, quercetin, myricetin, fisetin , and luteolin [4]. Phenol is a compound that dissolves in water easily [5]. Water and ethanol are polar compounds, water and ethanol are the types of solvents often used in the extraction process [6]. Onion oil is very active against a wide variety of bacteria [4]. Onions have been used in traditional medical practice to prevent and treat several diseases and disorders. Modern scientific research has shown that onions have antioxidant, anticancer, antibacterial, hypolipidemic, hypoglycemic, and anti-platelet aggregating activities [7].

Figure 1. Bulbs of Onion

Scientific classification (Onion classification) (8)
Class : Liliopsida
Sub-class : Lilidae
Family : Liliaceae
Genus : *Allium* L.
Species : *Allium cepa* L.

So far, no comprehensive article is available concerning the usage as an antibacterial compounds of *Allium cepa L.* By reviewing the literature on the antibacterial activity of water and ethanol extracts of *Allium cepa L,* has provided much information on its more precise uses. The aim of this review study was to identify relevant articles describing antibacterial activity of *Allium cepa* L. The present review articles are compiled in accordance with the extensive literature evidence on the antibacterial activity of *Allium cepa* L. We, therefore, considered it necessary to conduct a review to know what are the exact antibacterial effect of onion.

METHODS

The articles reviewed for this study were selected from the related publications in the last 10 years, ranging from 2010-2020BC. We performed an extensive literature search through PubMed, ScienceDirect, and Google scholar. The following descriptors or keywords used were (in all search fields): "Antibacterial" OR "Antimycobacterial" AND "*Allium cepa*" OR "Onion". No restriction was put in regard of language or by any type of publication. In articles evaluating the antibacterial activity of *Allium cepa L*, the data was collected for the zone of inhibition (ZOI), minimal bactericidal concentration (MBC), minimal inhibitory concentration (MIC), solvent, and the concentration and/or the dose. The most relevant articles were selected for further screening or evaluation.

RESULT AND DISCUSSION

Results presented in Table 1 are elaborated as below.

We analyzed twenty articles (Table 1). Almost all articles were searched for more than one type of bacteria. Nine articles reported about same bacteria. Fifteen articles reported about the zone of inhibition (ZOI), six articles reported minimal inhibition concentration (MIC) and two articles reported minimal bactericidal concentration (MBC).

The solvents used in all these articles are water and ethanol. Water and ethanol are the types of solvents often used in extraction [6]. Water is widely used because water is nontoxic, nonflammable, cheap, and widely available in pure form. In addition, it exists in a liquid state at relatively high temperatures (i, e., zero to 100°C) at one atmospheric pressure [9]. The difference of the solvent will have an impact on the antibacterial inhibition of *Allium cepa.* As reported by one of the studies, about the antibacterial effects of ethanol and water extract of *Allium cepa*, ethanol extract has a high inhibitory effect as compared to water extract against some bacteria, such as *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Proteus mirabilis* and *Salmonella spp. Escherichia coli* in ethanol extract have an inhibition zone diameter of 16 mm, while water extract have only 13 mm zone of inhibition [10]. As well as other studies showed that the ethanol extract of allium cepa is more effective in water extracts. A study has shown that *Escherichia coli* with 0.8 mg/ml in ethanol extract has 28 mm inhibition, while in water extract it has a 25 mm zone of inhibition [11]. Ethyl alcohol (ethanol) kills bacteria in two ways, protein denaturation, and fat membrane dissolution. Due to denaturation, the proteins in bacterial cells cannot work. As a result, an important processes in the bacterial cell are inhibited [12]. Destruction of bacterial cells is also possible through dissolving membrane lipids (fats), as the bacterial cell is surrounded by a lipid membrane. When ethanol is present, membrane lipids begin to be affected due to the presence of hydrophobic groups (CH3CH2) in ethanol. The hydrophobic groups and the lipid membrane begin to merge, but as a result, the protective forces of the lipid membrane begin to weaken and the work of the bacterial cell begins to be inhibited [13]. The difference in the structure of water and ethanol affects their activity against bacteria, ethanol activity against bacteria is greater than that of water, where ethanol has hydrophobic groups that can damage bacterial lipid membranes while in the water there are no hydrophobic groups.

The most widely studied bacteria in all the articles reviewed are *Staphylococcus aureus* and *Escherichia coli*. One of the researchers reported that *Escherichia coli* is easier to isolate and grow [14]. Other microorganisms that are tested are *B. subtilis, R. glutinis,S. cerevisiae, C. tropicalis, A. niger, M. purpureus, A. terreus, Vibrio cholera, B. cereus, B. licheniformis, Bacillus sp., S. mutans, K. pneumonia, S. typhimurium , P. aeruginosa , P. vulgaris , S. mareescens. E. coli* bacteria in 100µg concentration have the largest zone of inhibition compared to *K. pneumoniae, P. aeruginosa, S. aureus , E. faecalis, P. mirabilis,* and *Salmonella spp.[10]*. Other studies have also shown inhibition of *E. coli* bacteria have the largest zone of inhibition compared to Salmonella spp., S. pneumoniae, Shigella spp., and Staph. Aureus in 0.2 mg/ml concentration [11]. In 200/µg concentration *Allium cepa* can inhibit E. coli equal to 28 mm, on *Pseudomonas aeruginosa* gave 16 mm zone of inhibition, and *Staphylococcus aureus* showed a zone of inhibition of 23 mm diameter [15]. The *Allium cepa* extract used is made in various concentrations (Table 1).

The highest concentration is 50% for *S. aureus* with the maceration method and gave 16.03 mm ZOI (16). Another study reported that 25% of doses with Microwave-assisted extraction (MAE) gave a (ZOI) of 19.5 mm for *S. aureus* (17). This difference is possible due to the difference in the extraction method. Over other extraction methods, MAE has excellent advantages, such as higher yields, purity, and efficiency. In comparison with classic thermal extraction, the MAE proved to be a simpler but more effective procedure to obtain active compounds from medicinal plants [18].

Allium cepa L. has essential oil and is rich in phenolic compounds [5]. Some studies reported *Allium cepa* L. contains flavonoid, saponin, tannin, Ferulic acid, P-coumaric acid, alkaloids, organic acids, polyphenol, Cardiac Glycosides, and organosulfur compounds [19. 20].

Research from Gomma reported that *Allium cepa* in the form of deionized extract in 1.25 mg/ml concentration can inhibit E. coli [21]. Anyaegbunam reported that a dose of 50% of red onion extract could kill the E. coli bacteria, and 6.25% red onion extract could kill *Pseudomonas aeruginosa.* this is different from white onion, white onion cannot kill E. coli [22]

The higher the concentration given will have an impact on inhibition or killing of bacteria. high concentrations of extracts will cause many bacteria to die or have stunted growth, as reported by one study, 1 and 2.5% concentrations of the garlic extract have minor inhibition effects, at a concentration of 20 the bacterial inhibition was greater [23].

Table 1. The microorganisms used and the antibacterial activity of ethanol and water extract of *Allium cepa* L.

Solvent	Microorganism	Dose/ Concentration	Activity			Region	Ref. (s)
			MIC (mg/ml)	ZOI (mm)	MBC		
Ethanol extract (Allium cepa (purple and yellow))	*Vibrio cholerae*	Purple type 6.25%, yellow type 12.5%	EEP: MIC50 (19.2), MIC100 (21.6) EEY: MIC50 (67.2), MIC100 (68.4)			Pakistan	(24)
Water extract	*Escherichia coli*	0.5, 1, 2.5, 5, 10 and 20%,		Onion extracts showed no inhibitory effects against the E. coli		Iran	(23)
Deionized water extract	*B. subtilis* *B. cereus* *B. licheniformis* *Bacillus sp.* *S. aureus* *S. mutans* *E. coli* *K. pneumoniae* *S. typhimurium* *P. aeruginosa* *P. vulgaris* *S. mareescens*	0.078-10 mg/ml	*B. subtilis* (1.25) *B. cereus* (2.5) *B. licheniformis* (5) *S. aureus* (2.5) *S. mutans* (1.25) *E. coli* (1.25) *K. pneumoniae* (-) *S. typhimurium* (1.25) *P. aeruginosa* (1.25) *P. vulgaris* (-) *S. mareescens* (-)	*B. subtilis* (15) *B. cereus* (16) *B. licheniformis* (11) *S. aureus* (15) *S. mutans* (15) *E. coli* (11) *K. pneumoniae* (-) *S. typhimurium* (14) *P. aeruginosa* (15) *P. vulgaris* (-) *S. mareescens* (-)		Egypt	(21)
Water extract	*Staphylococcus aureus*	5, 10, 15, 20 dan 25 % b/v.		5 % (14); 10 % (15,5) 15 % (16) ; 20 % (19) ; 25 % (19,5)		Indonesia	(17)
Chloroform, ethanol and aqueous extracts	*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Proteus mirabilis* and *Salmonella spp*	100µg		• Ethanol: *E. coli* (16), *K. pneumoniae*(15), *P. aeruginosa*(9), *S. aureus* (14), *E. faecalis* (7), *P. mirabilis* (13), and *Salmonella spp* (10). • Aqueous: *E. coli* (13), *K. pneumoniae*(11), *P. aeruginosa*(10), *S. aureus* (8), *E. faecalis* (5), *P. mirabilis* (10), and *Salmonella spp* (9).		India	(10)
Water extract	*Pseudomonas aeruginosa*	25%		*Pseudomonas aeruginosa* (14)		Indonesia	(25)
Ethanol extract	*Staphylococcus epidermidis, Staphylococcus aureus, Salmonella thypi, and Eschericia*	50%, 25%; 12,5%, 6,25%, 3,125% , and 1,5625% b/v		• *S. aureus*: 50% (16.03); 25% (14.03); 12,5% (12.25); 6,25% (10.45); 3,125% (9.5); 1,5625% (8.97) • *S. epidermidis*: 50% (11.75); 25% (11.10); 12,5% (10.87); 6,25% (10.63); 3,125% (9.05); 1,5625% (8.55)		Indonesia	(16)

	coli.			• *S. thypi*: 50% (9.42); 25% (7.85); 12,5% (7.57); 6,25% (5.13); 3,125% (0); 1,5625% (0); • *E. coli.*: 50% (7.5); 25% (7.77); 12,5% (7); 6,25% (0); 3,125% (0); 1,5625% (0);			
Ethanol extract and water extract	*Escherichia coli, Salmonella spp., Streptococcus pneumoniae, Shigella spp.*, and *Staphylococcus aureus*	0.2 mg/ml		• Ethanol extract: 17.08 mm, 0.00 mm, 19.00, 19.00, and 15.0 mm for E. coli, Salmonella spp., S. pneumoniae, Shigella spp., and Staph. Aureus • water extract: 23.0 mm, 20.00 mm, 20.00 mm, 21.00 mm, and 21.00 mm for E. coli, Salmonella spp., S. pneumoniae, Shigella spp., and Staph. aureus		Nigeria	(11)
Ethanol extract	*Streptococcus pneumoniae*	20mg/ml 10mg/ml	• 10mg/ml concentration: 11.87 • 20mg/ml concentration: 18.62			Ethiopia	(19)
Water extract	*Staphylococcus aureus , Bacillus cereus, Escherichia coli and, Acetobacter aceti*		*Staphylococcus aureus* (31.3) , *Bacillus cereus* (125), *Escherichia coli* (125) *and, Acetobacter aceti*(31.3)			China	(20)
methanol extracts	*Listeria monocytogenes*	1 mg/ml	*Listeria monocytogenes* (125 µg /ml)	*Listeria monocytogenes* (11)	*Listeria monocytogenes* (500 µg /ml)	Iran	(26)
Water extract	*Pseudomonas aeruginosa, Staphylococus aureus* and *Escherichia coli*	0.1 ml	• Red onion: *Pseudomonas aeruginosa*,(3.125 % v/v) *Staphylococus aureus (-)* and *Escherichia coli* (50 % v/v) • White onion: *Pseudomonas aeruginosa*,(3.125 % v/v) *Staphylococus aureus (-)* and *Escherichia coli* (25 % v/v)		• Red onion: *Pseudomonas aeruginosa*, (6.25 % v/v) *Staphylococus aureus (-)* and *Escherichia coli* (-) • White onion: *Pseudomonas aeruginosa*, (6.25 % v/v) *Staphylococus aureus (-)* and *Escherichia coli* (50 % v/v)	Nigeria	(22)
Ethanol	*Staphylococcus*	50 µl, 100 µl, and		*E. coli* with a zone of inhibition of 28 mm (at		India	(15)

extract	*aureus, Pseudomonas aeruginosa* and *Escherichia coli*	500 µl	conc. 200/µg) and was least effective against *Pseudomonas aeruginosa* with zone of inhibition of 16 mm (at conc. 200 µg.). *Staphylococcus aureus* showed a zone of inhibition of 23 mm diameter (at conc. 200 µg.).		
Water extract	*Propionibacterium acnes*	5%, 10%, 20% and 40%.	5% (12,8 mm), 10% (13 mm), 20% (14,33) dan 40% (15,50 mm)	Indonesia	(27)
Ethanol extract	*E .coli, P. mirabilis S. typhi P. aeruginosa*	50 mg/ml	*E .coli* (19), *P. mirabilis* (22.5), *S. typhi* (19.5), *P. aeruginosa* (16.5)	Nigeria	(28)
Ethanol extract	*Staphylococcus aureus*	5%b/v, 10%b/v, 20%b/v, 40%b/v, 60%b/v, 80%b/v.	5%b/v (7), 10%b/v (8.30), 20%b/v(9.60), 40%b/v(11), 60%b/v(12.33), 80%b/v(14.33).	Indonesia	(29)
Water extract	*Enterobacter aerogenes, Escherichia coli , Salmonella enteritidis , Salmonella typhimurium, Staphylococcus aureus* , and *Bacillus subtilis*	(800, 400, 200 and 100 mg/mL	• White onion : *Enterobacter aerogenes* : 800 mg/mL (40), 400 mg/mL(34.33), 200 mg/mL (17.33), and 100 mg/mL (0) ;*Escherichia coli*: 800 mg/mL (26.33), 400 mg/mL(18.33), 200 mg/mL (16.33), and 100 mg/mL (0); *Salmonella enteritidis*: 800 mg/mL (31.67), 400 mg/mL(26.33), 200 mg/mL (21.67), and 100 mg/mL (0) ; *Staphylococcus aureus*: 800 mg/mL (21.67), 400 mg/mL(16.33), 200 mg/mL (0), and 100 mg/mL (0);*Bacillus subtilis*: 800 mg/mL (0), 400 mg/mL(0), 200 mg/mL (0), and 100 mg/mL (0). • Red onion : *Enterobacter aerogenes* : 800 mg/mL (22.67), 400 mg/mL(15.67), 200 mg/mL (11), and 100 mg/mL (0) ;*Escherichia coli*: 800 mg/mL (26.33), 400 mg/mL(18.33), 200 mg/mL 16.33(), and 100 mg/mL (0); *Salmonella enteritidis*: 800 mg/mL (33), 400 mg/mL(26.33), 200 mg/mL (16.33), and 100 mg/mL (0) ; *Staphylococcus aureus*: 800 mg/mL (22.67), 400 mg/mL(21.67), 200 mg/mL (18.67), and 100 mg/mL (0);*Bacillus subtilis*: 800 mg/mL (0), 400 mg/mL(10), 200 mg/mL (0), and 100 mg/mL (0).	Turkey	(30)
Ethanol extract	*Shigella flexneri, Enterococcus faecalis, Staphylococus aureus, Proteus mirabilis, Salmonella typhi, Serratia marcescens,*	11.6%	*Shigella flexneri* (5), *Enterococcus faecalis* (3.75), *Staphylococus aureus* (7.5), *Proteus mirabilis*(3.75), *Salmonella typhi*(0), *Serratia marcescens* (1.87), *Klebsiella pneumonia* (1.87), *Escherichia coli* (7.5), *Pseudomonas aeruginosa* (5).	India	(31)

	Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa				
Water extract	*Aeromonashydrophila*	60 mg/m	Only hot – water extract of white Allium cepa (onion) showed antibacterial activity against Aeromonashydrophila isolates	Iraq	(32)
Ethanol extract	*staphylococcus aureus, bacillus subtilis, escherichia coli, salmonella typhimurium* and *enterobacter aerogenes*		Mature bulb: *staphylococcus aureus* (12.6 mm), *bacillus subtilis* (12.7 mm), *escherichia coli* (11.2 mm), *salmonella typhimurium* (3.6 mm) and *enterobacter aerogenes* (11.9 mm)	Pakistan	(33)

ZOI: Zona of Inhibition; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration.

CONCLUSION

Ethanol extract have a high inhibitory effect as compared to water, against some bacteria, such as, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Proteus mirabilis,* and *Salmonella spp.* The higher the concentration used, the stronger will be the inhibitory, or killing effect on bacteria. High concentrations of extracts will cause many bacteria to die or induces a stunted growth. Different extraction methods affect the antibacterial activity of *Allium cepa, L*. Different types of *Allium cepa*, affects its antibacterial activity. The antibacterial activity of the red onion is higher than white onion

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