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Research article

Horseradish Seeds and Sodium Bicarbonate Efficacy against Clinically Isolated *Vibrio cholera in vitro*

***Ali Abedulameer Alhusayni* [1], *Falah Hasan Obayes AL-Khikani* [1,2]**
1. *Department of Microbiology, Al-Shomali General Hospital, Babylon Health Directorate, Babylon, Iraq*
2. *Department of Medical Laboratory Technology, College of Medical Technology, Islamic University, Najaf, Iraq.*

ABSTRACT

Background*: Vibrio cholera* causes a severely lethal secretory diarrheal disease. It is a gram-negative, motile, curved-rod bacterium. Cholera, an acute diarrheal disease, poses a serious threat to public health, particularly in developing countries. Horseradish (*Armoracia rusticana*) is a large-leaved, hardy perennial plant. It is used today primarily as a condiment, however, has also been known as a medicinal herb. **Material and methods***: Vibrio cholera* O1 Ogawa serotype was isolated from fifteen Iraqi patients. Isolates were obtained from watery diarrheal stool then cultured in peptone water for 8 hrs and were cultured overnight on blood agar, MacConkey agar and TCBS Agar, then they were biochemically tested. Horseradish and *Sodium bicarbonate* extracting preparation was 2.5% and 2% respectively by working powder from seeds and dissolving distilled water then putting the solution in autoclave 120 ℃ fore 20 min. The antibiotic susceptibility testing was done by the standard well diffusion method on Mueller-Hinton agar. **Results***:* Horseradish seeds showed 93% activity against 15 *Vibrio cholera* O1 Ogawa serotype, Tetracycline revealed 82% activity and Trimethoprim was 46.6% active. Sodium Bicarbonate was ineffective 100% against *Vibrio cholera*. Mean comparison of activity of Radish seeds 18.47 mm was non- significant compared to Tetracycline 19.27 mm (P= 0.74) while it was significant when compared to Trimethoprim 12.20 mm (P= 0.008)**.** No correlation was observed between Radish seeds and both tetracycline and trimethoprim (p> 0.05). **Conclusion***:* Horseradish seeds are promising novel antibacterial agent for *V. cholera* treatment; clinical studies *in vivo* are demanded.

Keywords:
Vibrio cholera O1, Horseradish seeds, Armoracia rusticana, Sodium Bicarbonate, Trimethoprim, Tetracycline.

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*** Corresponding author:**
Ali Abedulameer Alhusayni
E-mail address:
alti99269@gmail.com

INTRODUCTION

The gram-negative, motile, curved-rod bacterium *Vibrio cholera* causes the severe, fatal secretory diarrheal illness cholera. There are more than 200 serogroups of *V. cholera* [1]. Its adhesion to the small intestine and colonization by lipid A and flagella enable bacterial activity. Following proliferation, the release of heat-sensitive exotoxin activates the intestinal mucosa, causing the cells to disrupt the exchange of sodium ions. This results in the large-scale release of water and electrolyte into the gut, which lowers blood plasma levels and ultimately causes death [2].

Acute diarrheal illness, or cholera, is one of the major dangers to public health worldwide, especially in underdeveloped nations, where it accounts for 1.4–4.3 million reported cases and 28,000–142,000 fatalities annually. Cholera outbreaks are often brought on by the cholera toxin-producing *Vibrio cholera* O1 and O139 bacteria, and they are most likely to occur in congested places with insufficient sewage treatment, water and sanitation facilities, and poor hygiene practices. The consumption of tainted food and water from

domestic or environmental sources is the primary cause of cholera outbreaks [3]. *Vibrio cholera* may become more transmissible when it passes through the human gut, perhaps leading to an increased risk of epidemics. *Vibrio cholera* produced *in vitro* has a higher infectious dose (about one-fifth) than the infectious dosage of the bacteria lost in human feces in a hyper-infectious condition [4].

Horseradish (*Armoracia rusticana*), a large-leaved, hardy perennial plant in the *Brassicaceae* family, is mostly composed of glycosinolates (GSLs) [5]. Different chemical structures, such as isothiocyanates (ITCs), thiocyanates, nitriles, epithionitriles, and oxazolidinethiones, are produced as GSLs degrade [6-7]. Three methods can be used to start GSL degradation: chemically, thermally, or enzymatically. It is indigenous to Western Asia and southeast Europe [8]. Although it has long been recognized as a medicinal herb, it is presently mostly used as a condiment [9–10].

Sodium Bicarbonate (SB) is widely used in foods at levels up to 2% for leavening, pH-control, taste, and texture development [11]. There is a dearth of information about SB's antibacterial capabilities. It is employed in dental preparations and inhibits periodontal pathogens [12]. *Aspergillus parasiticus* is killed by SB, which also modifies the distribution of aflatoxin in cells that survive. Its low cost and non-toxicity make it a good choice for a preservative [13]. The aim of this investigation was to ascertain if horseradish and SB have antibacterial properties against *V. cholera*.

MATERIALS AND METHODS:

Vibrio cholera O1 Ogawa serotype were isolated from fifteen Iraqi patients in Al- Shomali General Hospital, Babylon city, Iraq, from1/3/2022 to 20/10/2022. Isolates were obtained from watery diarrheal stool then cultured in peptone water for 8 hrs and were cultured overnight on blood agar, MacConkey agar, and TCBS agar to distinguish between different species, then they were biochemically tested by using oxidase, indole, urease, simon citrate, kligler iron agar, triple sugar iron agar, Gram stain, string test, and antisera polyvalent; Ogawa and Inaba. Isolation and identification were performed according to standard methods [14].

Horseradish extracting preparation was 2.5%, by working powder from seeds and dissolving 2.5grams of horseradish seeds powder in 100 ml distilled water then putting the solution in autoclave 120 ℃ fore 20 min. *Sodium bicarbonate* was 2%, by putting 2grams from powder in 100 ml distilled water and then it was put in autoclave 120 ℃ fore 20 min.

Mueller-Hinton agar was used for the classic well diffusion method of assessing antibiotic susceptibility. The Clinical and Laboratory Standards Institute (CLSI) standards [15] were followed to achieve a homogenized bacterial inoculum solution using turbidity standard technique. To evaluate the antimicrobial activity of Horseradish extracts and *Sodium bicarbonate*; the agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 mm is punched with a sterile tip, and a volume (50 µL) of the Horseradish was introduced into the wells. *Sodium bicarbonate* was introduced in the same method. Then, agar plates were incubated under suitable conditions. The disc diffusion method was used to evaluate the activity of tetracycline (30 µg) and trimethoprim (5 micro g).

SPSS program 26 (SPSS Inc., Chicago, USA) was utilized for statistical analysis. To depict the data, means and standard deviations were employed. Measurement data were examined using the T-test. The correlation between two variables is assessed using Spearman's correlation. $P < 0.05$ is regarded as significant.

RESULTS:

This study shows that most mothers are urban (53%) and rural (47 %), according to their employment status; the results indicate that the majority were unemployed (95). Horseradish seeds showed antibacterial activity against (93%) of investigated *Vibrio cholera* serotype o1 while 7% were resistant, tetracycline was 82% sensitive, 12% resistance and 8% intermediate, whereas trimethoprim was 46.6% sensitive and 53.4%

resistant. Sodium Bicarbonate revealed no effective against *Vibrio cholera* [Figure 1].

The radish seeds sensitivity mean of inhibition zone 18.47 mm was smaller than tetracycline 19.27 mm (P= 0.74). Radish seeds sensitivity mean of the inhibition zone was more than trimethoprim12.20 mm significantly (P= 0.008) [Table 1].

No Correlation was observed between radish seeds and tetracycline activity (p=0.415). Moreover, there was no correlation between radish seeds and trimethoprim activity (p=0.383) [Table 2].

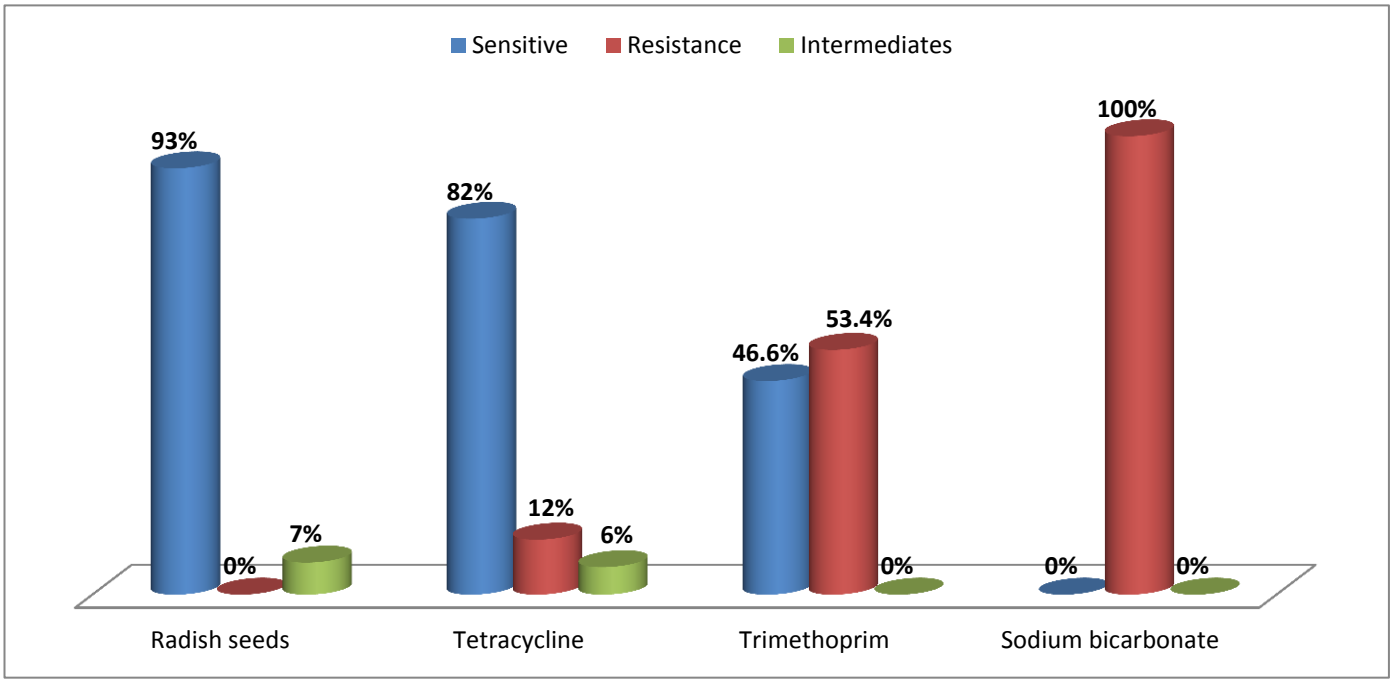

Figure 1: Antimicrobial activity of different agents

Table 1: Mean comparison of different agents

Variables	Mean	No.	Std. Deviation	*P* value
Radish seeds	18.47	15	4.642	0.74
Tetracycline	19.27	15	6.964	
Radish seeds	18.47	15	4.642	**0.008**
Trimethoprim	12.20	15	7.504	

Table 2: Correlation between different agents

Variables	No.	Correlation	Significance
Radish seeds and tetracycline	15	- 0.227 -	0.415
Radish seeds and trimethoprim	15	0.243	0.383

DISCUSSIONS:

Horseradish (*Armoracia rusticana*), a large-leaved, hardy perennial plant in the *Brassicaceae* family, is mostly composed of GSLs. Although it has long been recognized as a medicinal herb, horseradish is currently mostly used as a condiment [16].

Horseradish seeds (*Armoracia rusticana*) in this study showed high activity against *Vibrio cholera* which was 93% sensitive and 7% resistant, for tetracycline was 82% sensitive, 12% resistant and 8% intermediate, and for trimethoprim was 46.6% sensitive and 53.4% resistant. These findings agree with another study that found low resistance to

tetracycline 6.4% and high resistance to trimethoprim 99.1% [16]. Another study establishes low levels of resistance to tetracycline (6.2%) and trimethoprim (96%) [17]. Another study described similar patterns of resistance to tetracycline (11%) [18]

About 90% of the isothiocyanate (ITC) extract from horse radish and 9% of 2-phenethyl isothiocyanate were present in the distillate utilized in another study. For seven days under aerobic storage, the growth of *Salmonella typhimurium*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, and S*erratia grimesii* on agar was entirely prevented. Different species exhibited varying levels of bactericidal action, which rose with distillate concentration. Only three bacteria were found to exhibit bactericidal activity: *Serratia grimesii*, *Escherichia coli*, and *Staphylococcus aureus* [19].

The results of this analysis corroborated those of a previous study that reported the antibacterial properties of ITCs isolated from horseradish root. *Staphylococcus aureus* was shown to be inhibited by ITCs at concentrations of more than 2,000µg/mL. The inhibitory zones against *S. aureus*, *A. baumanii*, and *P. aeruginosa* were measured to be 55, 32, and 85 mm, respectively [20].

Stoin and colleagues used the inoculate microbiological technique on the surface of culture medium to test the antibacterial activity of ITCs extracted from horseradish (*Armoracia rusticana*) against the following microbiological cultures: *Escherichia coli, Candida albicans, Bacillus subtilis, Staphylococcus aureus, Agrobacterium tumefaciens,* and *Rhizopus nigricans*. The inhibitory activity of the ITCs on *Bacillus subtilis* is mediocre. When extracts from mustard seeds are used, *Candida albicans* show a notable sensitivity to the effect of ITCs; at maximal concentrations, the free zone width is 0.7 cm, and it declines to the other values, remaining unchanged for 48 hours [21].

This study revealed that Sodium Bicarbonate has no effect against *Vibrio cholera*. SB is widely used in foods at levels up to 2% for leavening, pH-control, taste, and texture development. Data on the antimicrobial properties of SB are limited. It is inhibitory to periodontal pathogens and is used in dental preparations [22].

This result is consistent with another study's conclusion that sodium bicarbonate is ineffective against *Vibrio cholera*. A novel medium was created to encourage *Vibrio cholerae* O1 El Tor Biotype (El Tor vibrios) to produce cholera toxin *in vitro*. 0.5% NaCl, 0.3% NaHCO3, 0.4% yeast extract, and 1.5% Bacto-Peptone are all included in the medium. El Tor vibrios were cultivated for 20 hours at 37°C in a stationary test tube. Using a reversed passive latex agglutination approach, the cholera toxin content of the culture supernatant was measured. Compared to standard syncase media, the majority of vibrios cultivated in this medium generated 10–20 times as much toxin [23].

CONCLUSION

Horseradish seeds are promising novel antibacterial agent for *V. cholera* treatment; clinical studies *in vivo* are demanded.

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