CHAPTER I

INTRODUCTION
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Introduction

1.1 General.

*Vibrio cholerae* discovered by Koch in 1883 is a human pathogen. The evidence adduced to prove that epidemic, or as it is commonly called Asiatic cholera, a specific infection caused by the *Vibrio cholerae* was present in ancient India has been differently evaluated by different writers. Some of those in favour of an early existence of the disease pointed to descriptions of a syndrome showing clinical features, identical with those of true cholerae in the ancient Indian medical literature particularly in the writings of Susruta.

Cholerae infection appeared at the end of October 1819 in Mauritius, between Arabia and East African Coast (Haeser, 1882, Hirschi, 1883). The wave of infection reached Berlin (1831) and England in 1831 (Macnamara, 1876). Cholera appeared in Belgium in the spring of 1832. In Norway in the autumn of 1832 (Hirsch, 1883). Cholera reached the distant shores of America in 1832 (Haeser, 1882 and Hirsch, 1883). Cholera was imported into Portugal early in the year 1833 and penetrated into Spain in the same year.

Out break of cholera took place in Meeca in 1865 and then the infection was carried out to other parts of Arabia,
Mesopotamia, Syria and Palestine, Alexandria, Egypt, Istanbul. From Istanbul (1865) the infection was spread over to Turkey as well as southwards to Asia Minor, Cyprus, Bulgaria, Rumania, Austria, Russia.

The infection became serious in Italy and also became fairly wide spread in France. Besides ranging in Europe and America, cholera showed an extensive spread in Africa in 1864-65. During the period 1865-70 cholera became epidemic in several West Indian Islands.

*Vibrio parahaemolyticus* is a human pathogen causing severe gastroenteritis. It is a gram negative organism and has proved to be the leading cause of bacterial food poisoning amongst the Japanese for more than two decades. This organism was first discovered by Fujino of Japan in 1951 (Fujino et al., 1951). It was classified as vibrio species in 1963 after a comprehensive study on over 1700 isolates was carried out by Sakazaki et al., in 1963. Recently *V. parahaemolyticus* has been recognised as an important agent of diarrhoeal disease all over the world. Within the last few years, this organism has been isolated viz. north west pacific (Baross and Liston, 1969), Washington state (Baross and Liston, 1970), gulf coast (Vanderzant, et al., 1970), South East Asia (Saito, 1970), Netherlands (Kapelmacher, et al., 1970, 1972), New Hampshire (Bartley and Shanetz, 1971),

These reports indicate that *V. parahaemolyticus* is widely distributed throughout the world.

In India, this organism was first isolated in Calcutta from cases of acute diarrhoea and dysentery during 1966-1969 (Neogy, 1970; Neogy, et al., 1970, Chatterjee, et al., 1970a, 1970b).

The bactericidal property of essential oil had been known for a long time. Essential oils were used with some success for the treatment of cholerae. It is of importance to note that in the experience of some workers such as Babes (1885) and Riedlin (1888) such oils were found capable of inhibiting the growth of *V. cholerae*. The former of these observers found mustard oil was far more effective in this respect than peppermint oil, turpentine oil and oil of thyme.
Both Sealy (1922) and Tomb (1923; 1926) pointed out that the use of essential oil had been known and used with apparent success by layman in India for many years.

In our indigenous system of medicine too some plant products were considered to be "specific remedies" against cholera (Sengupta, 1919).

De and Subramanyan (1930) studied the relative bactericidal values of a number of Indian essential oils and their constituents.

The great value of treatment with essential oils in the early stage of cholera was endorsed by a number of subsequent workers, for instance, by Yui (1925), Bharati (1926), Lico (1926), Cannon (1927), Morison, Rice, and Haytharnthwaite (1934). Megalle, et al. (1980) found that some essential oil constituents have antimicrobial activity.

The compounds which were selected for studying their action on *V. cholerae* and *V. parahaemolyticus* are menthol, diosphenol, cyclohexane, cyclohexanol, cyclohexanone, benzene, thymol, carvacrol (isothymol); p-cymene, o-cresol, m-cresol and p-cresol, resorcinol, benzylamine, vanillin. Of these menthol, diosphenol, thymol, carvacrol (isothymol) and p-cymene are constituents of different essential oils - which were used against *V. cholerae*. 
Materials such as thymol, menthol, etc., which have been used for many years for inhibition therapy in respiratory disorders are shown to liberate vapours which are bactericidal for the types of organisms associated with such infections studied by Thomas C. Grubb (1959).

1.2 Action of menthol on bacterial cells

The growth inhibitory effect of camphor has been reported in *V. cholerae* (Adhikari, 1975) and in *V. parahaemolyticus* (Adhikari, Chakrabarti and Adhikari, 1976). They reported that camphor at suitable concentration inhibits the biosynthesis of protein, RNA and DNA in *V. cholerae* and *V. parahaemolyticus*.

Camphor is a bicyclic terpene. The monocyclic terpene menthol (or-3-hydroxy-4-isopropyl-1-methyl cyclohexane) is the most important terpene alcohol of the menthane series. It occurs in the oil of peppermint. Menthol is of value as a counterirritant in neuralgia and headache, and its vapours from nasal inhalers is used to give relief from cold and bronchitis.

Petrovski (1972) studied the effect of some essential oils on *V. cholerae* and *V. parahaemolyticus* and found that peppermint oil was bactericidal in distance but bacteriostatic on direct contact.
Avramov et al. (1965) studied the action of a mixture containing chlorophenol, camphor, menthol and acetone on Staphylococcus aureus.

The action of menthol on V. cholerae was studied by Nayak and Dutta (1961).

They studied the effect of oils of cardamom, clove, cajaput, junipar, peppermint, lemon grass, zanthoxylum budrunga and three chemicals namely thymol, menthol and camphor. Only sterile oils were used by them in the experiments. Oils were then dissolved in a 2% sterile tween 80 solution. Varying quantities of the oil solutions were added to the casein hydrolysate medium. The total volume of the medium was 10 cc and kept as constant. They used rabbit passaged strain of V. cholerae (Inaba, 569-B) as the test organism. The potency of the oils and the drugs was tested against $10^4$ vibrios per cc. by then. They preserved the rabbit passaged strain of V. cholerae in a lyophilised state and regenerated before each experiment. It was suitably dilutered and finally diluted to contain $10^4$ vibrios per 100 g of rabbit body-weight. They administered the vibrio suspension intraintestinally to infant rabbits 10 to 12 days old. They started the treatment by administering the oils orally one hour before inoculation of the vibrio followed by further administration at six hourly intervals.
1.3 Action of diosphenol on bacterial cells.

Diosphenol (1-methyl-4-isopropyl cyclohexen-2-ol-3-one) is a monocyclic terpene and is obtained from the leaves of various species of Barosma, B. betulima, Partil, B. serratifolia Willd.

It was synthetically prepared by Semmler and Mc Kenzie by the oxidation of hydroxy methylene menthone, the diketone so obtained tautomerising to the hydroxyketone (The terpenes - J.L. Simonson). It is structurally related to the monocyclic terpene menthol. It is one of the components of mint oil-known to have antibacterial effect.

1.4 Action of cyclohexane on bacterial cells.

Cyclohexane the hydroaromatic compound is the parent compound of menthol and diosphenol. This cyclohexane on oxidation produces cyclohexanol and cyclohexanone.

1.5 and

1.6 Action of cyclohexanol and cyclohexanone on bacterial cells.

These two hydroaromatic compounds are structurally related to the monoterpenic menthol.
Lindenberg and Massin (1957) studied the action of cyclohexanol on *Salmonella typhosa* and *Saccharomyces cerevisiae*.

1.7 **Action of benzene on bacterial cells.**

Benzene is an aromatic compound. The aromatic compounds are all related to benzene - the parent hydrocarbon with six carbons having alternate single and double bond. The aromatic compounds are exclusively cyclic. So it is the parent compound of thymol, carvacrol (isothymol), p-cymene, cresols, resorcinol, benzylamine, vanillin. It is also structurally related to the hydroaromatic compound cyclohexane. Benzene is somewhat toxic substance in the liquid or vapour state.

1.8 **Action of thymol on bacterial cells**

AND

1.9 **Action of carvacrol (isothymol) on bacterial cells.**

Thymol and carvacrol (isothymol) are isomeric phenols derived from p-cymene. Thymol occurs in the oils of thyme and mint and ajowan and carvacrol (isothymol) is found in oil of caraway. Both substances have pronounced germicidal action (organic chemistry by Ray Q. Brewster and William E. McBven).
Lindenberg and Massin (1957) studied the bactericidal action of thymol on *Salmonella typhosa* and *Saccharomyces cerevisiae*.

Mikhanovaskaya *et al.* (1958) studied the fungicidal properties of carvacrol (isothymol) against *Trichophyton gypseum, F. violaceum, Candida albicans* and *C. etellatoides*. The action of thymol on *V. cholerae* was studied by Nayak and Dutta (1961) (Details of the experiment was described in section 1.2). They also reported that Bhaskaran, Chatterjee and Tiwaree (1958) have reported on the marked vibriolytic property of thymol.

Jong Hyup Kim *et al.* (1964) studied the fungicidal activity of thymol against *Aspergillus niger*. Oka (1964) studied the action of thymol on *E. coli* and *Staphylococcus aureus* and on yeast cells.

1.10 **Action of p-cymene on bacterial cells.**

The two germicidal phenols – thymol and carvacrol (isothymol) are derived from cymene. The mono cyclic terpenes are also related to the aromatic hydrocarbon p-cymene. Both the phenols and monocyclic terpenes have antimicrobial action. Again p-cymene is structurally related to toluene which is a growth inhibitor of *V. parahaemolyticus*.
p-cymene is a constituent of essential oils of citrus fruits.

1.11 Action of cresols (Ortho, meta and para) on bacterial cells.

Weuffen, et al. (1970) synthesised seventy three phenols and tested for activity against pathogenic bacteria and fungi and showed that methyl, halogen and nitro substitution increased microbicidal activity. So the cresols have bactericidal property. The cresols are structurally related to thymol and carvacrol and also p-cymene.

1.12 Action of resorcinol on bacterial cells.

Weuffen, et al. (1970) synthesised seventy three phenols and tested for activity against pathogenic bacteria and fungi. They found that hydroxyl group substitution decreased activity when compared to unsubstituted phenols.

Lindenberg and Massin (1957) studied the action of resorcinol on Salmonella typhosa and Saccharomyces cerevisiae.

1.13 Action of benzylamine on bacterial cells.

Benzylamine is structurally related to phenethyl alcohol. The bacteriostatic action of phenethyl alcohol (PEA) was first
reported on *E. coli* by Lilley and Brewer (1953) and also on *V. parahaemolyticus* by Adhikari, et al. (1977).

1.14 *Action of vanillin on bacterial cells.*

Vanillin is an aromatic compound structurally related to benzene. Katayama and Nagai (1960) studied the activity of vanillin against *Bacillus subtilis, Salmonella enteritidis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus morganii*.

1.15 *Scope of the present work.*

All the selected compounds are structurally related to one another.

![Menthol](image1)

![Diosphenol](image2)

![Cyclohexane](image3)
Cyclohexanol

Cyclohexanone

Benzene

Thymol
Carvacrol (isothymol)

p-Cymene

o-Cresol
m-Cresol
p-Cresol

Resorcinol
Benzylamine
Vanillin
Menthol or -3-hydroxy 4-isopropyl-1-methyl cyclohexane and diosphenol or 1-methyl 4-isopropyl cyclohexen-2-ol-3-one is related structurally to cyclohexane—the hydroaromatic hydrocarbon. Cyclohexanol and cyclohexanone are produced by oxidation of cyclohexane. This hydroaromatic hydrocarbon (cyclohexane) and the aromatic hydrocarbon benzene with alternate double bond contain the same number of carbon atoms and both are cyclic. Benzene is the parent hydrocarbon of all aromatic compounds. Thus all aromatic compounds are benzenoid compounds. All the compounds used in this study except the monocyclic terpenes (menthol and diosphenol) and hydroaromatic compounds (cyclohexane, cyclohexanol and cyclohexanone) are aromatic.

The monocyclic terpenes are related to aromatic hydrocarbon p-cymene. Thymol and carvacrol (isothymol) — the two isomeric phenols are derived from cymene.

All the cresols are also structurally related to p-cymene and resoreinol. Benzylamine, vanillin are also derivatives of benzene.

These compounds have been selected carefully with the object of finding out the structural relationship of these compounds with regard to their effect on the growth and activity of *Vibrio cholerae* and *V. parahaemolyticus*. 

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The action of these cyclic and aromatic compounds have been studied on these two pathogenic organisms. The mode of action of these agents on the two bacteria *V. cholerae* and *V. parahaemolyticus* are likely to enrich our present day knowledge. The recorded literature does not contain any significant information in this particular field of study.