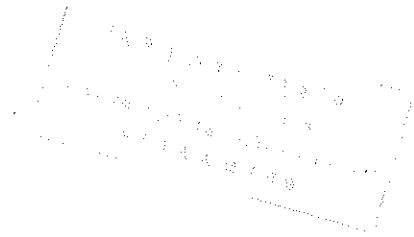


**ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES**

***IN-VITRO* ANTIMICROBIAL ACTIVITY OF SOME
TRADITIONAL MEDICINAL PLANTS ON SOME
FOOD BORNE PATHOGENS**

BY



MINTESNOT ASHEBIR

JUNE, 1998

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FOOD BORNE PATHOGENS**

**A THESIS SUBMITTED TO
THE SCHOOL OF GRADUATE STUDIES**

**IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER
OF SCIENCE IN BIOLOGY**

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JUNE, 1998

ACKNOWLEDGEMENT

Above all, I would like to say that I have no words to express my deepest and heartfelt gratitude to my advisor Dr. Mogessie Ashenafi. His assistance, encouragement and punctuality in all time when I need his assistance throughout this work would never be forgotten.

I would also like to thank, Dr. Abera Giyied who have kindly given me the test organisms. The visit, advice and encouragement of Ato Gashaw Mamo, the assistance of Ato Admassu Bimerew in providing me all materials that I need in time is dully acknowledged.

I would also never forget the time that I have passed with my friends, Feleke Moges, Solomon Zedie, Sineshaw Aysheishem and W/t Zenebech. Their assistance is also highly acknowledged.

I would also appreciate the help of Mamie and Almaz.

Last but not least my deepest gratitude also goes to my sister Gete Nigatu who really helped me more than she must do for me. Finally I would thank all friends who collaborated me.

The financial support of the Swedish Agency for Research Co-operation in the developing Countries (SAREC) is greatly acknowledged.

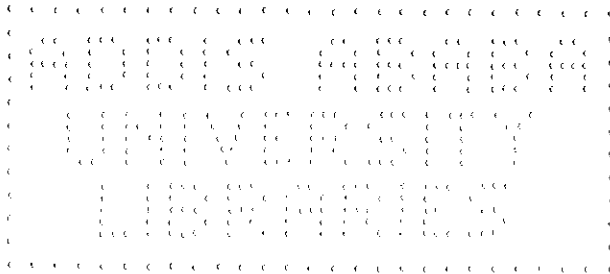


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ABSTRACT

Eleven various types of traditionally used Ethiopian medicinal plants have been screened for their antimicrobial activity at the crude preparation level against six different bacteria, two Gram +ve (*Bacillus cereus* and *Staphylococcus aureus*) and four Gram -ve (*Escherchia coli*, *Shigella boydii*, *Shigella flexneri* and *Salmonella typhimimum*). Of the tested crude preparations only two (*Ruta chalepensis* and *Echinops* spp.) showed antimicrobial activity against *B. cereus* by the agar well-plate diffusion assay. The effects of these crude preparations were found to be heat dependent. Eight of the tested crude preparations at various concentrations by agar dilution assay were found to be effective in or both of the Gram positive bacteria at various concentrations, as assessed by agar dilution assay. The MIC of *Syzygium guineense* (0.312%) was the same for all susceptible organisms (*B. Cereus*, *S. aureus*, *Sh. flexneri* and *S. boydii*). It appeared to be bacteriocidal for *S. aureus*, *Sh. flexneri* and *S. Boydeii*. It appeared to be bacteriocidal for *S. aureus*, *Sh. flexneri* and *S. boydii* and bacteriostatic for *B. cereus*. All the active preparation found effect by agar dilution assay, were found to be stable upon heating upto 121° C for 15 minutes.

The MIC of *Carum copiticm*, *Ruta chalepensis* and *Artemisia afra* against *B. cereus* was 5%. It was higher for *Cymbopogon citratus* (10%) and lower for *Echinops* spp.(0.625%) and *Syzygium guineense* (0.312%). The MIC of *Vernonia amygdalana* was 5%. For *S. aureus* the MIC for the latter two plants was the same as for *B. Cereus*.

Of the tested crude preparations, only *Syzygium guineense* appeared to be bacteriocidal. The MBC was 1.25% for *S. aureus* and 5% for *Sh. flexneri* and *S. boydii*. The crude preparations at the MBC killed all the population within 8 h in all the above mentioned organisms.

Broth dilution assay was conducted to assess the antimicrobial effect of the crude preparations concentrations: *Carum copiticum*, *Coriandrum sativum*, *Echinops* spp. at 10%; *Ruta chalepensis*, *Foniculum vulgare*, *Cymbopogon citratus*, *Vernonia amygdalina* and *Ginger officinale* at 7%; *Artemisia afra* at 5% and *Lipidium sativum* at 2%. The results showed that eight of the crude preparations have reduced the final population of *B. cereus* by one to four log units as compared to the control. On the other hand, ten of them were found to reduce the final population of *S. aureus*. Only one of the crude preparations had retarding effect on *Sh. flexneri*, and two on *Sh. boydii*. None of them has inhibitory effect on *E. coli* and *Salmonella typhimurium* except *Syzygium guineese* that decreased the final population of *Salmonella*.

1. INTRODUCTION

Food borne diseases are generally of two types. Those that are caused by ingesting the actual etiologic agent of the disease, such as *Salmonella*, are called food infections, whereas those caused by consuming chemical agents like arsenic, cadmium or a poisonous organic plants and animals (such as mushrooms, calms or toxins (staphylococcal, botulinal), present in a food are called food poisoning (Tartakow and Vorperian, 1981).

Food borne illness is a significant public health problem ranging from acute gastroenteritis which kills millions of children in poor countries to the diarrhea suffered by the affluent air traveller; from trichinellosis due to eating fermented pork to illness caused by chemicals contaminating food (Evans and Feldman, 1984; WHO, 1991).

Contaminated food has been the case of human illness since antiquity. The agents are diverse. They can be of either biological or non-biological origin. The non-biological agents are agents like pesticides, additives and heavy metals. Whereas the biological agents can be bacteria, viruses, fungi or animal parasites.

There are a wide variety of bacteria identified as the cause for food borne illness. Evans and Feldman (1984) in USA have identified about nine bacteria:- *Bacillus cereus*, *Clostridium perfringens*, *Vibrio parahemolyticus*, *Staphylococcus aureus*, *Clostridium botulinum*, *Vibrio cholerae*, toxigenic *Escherichia coli*, *Salmonella* and

Shigella as major recognized etiologic agents and *Yersinia enterocolitica*, *Campylobacter fetus* sub spp. *jejuni*, *Streptococci* and *Arizona* spp. as infrequently isolated agents.

Food borne diseases caused by bacteria remains a serious problem in all countries and most of them can cause diarrhea. Up to 70% of all episodes of diarrhea may result from the ingestion of contaminated food or water (Esrey and Feach, 1989 cited in Abdussalem and Kaferstein,1994). These diarrheal diseases are major causes of morbidity, with attack rates ranging from 2 to 12 or more illness per person per year in developing countries (Gurrant *et al.*,1990). In tropical developing countries, it is the cause of morbidity as well as a leading cause of mortality. About 4.6 million deaths per year or 12,600 deaths/day in children in Asia, Africa and Latin America is due to diarrheal diseases(Snyder and Merson, 1982).

Industrialized countries with their well developed health care systems and strong infrastructure of legislation, standards and enforcement mechanisms, have tried to control food borne infection and intoxications. Their effort have been strengthened by supply of safe piped water, improved sanitation, better facilities for personal hygiene and wide application of food safety techniques such as pasteurization and, to some extent, irradiation. These measures have helped to eliminate or reduce some diseases such as cholera, typhoid fever, bacterial dysentery, but not the overall incidence of food borne diseases. Indeed, some, such as none typhoid salmonellosis and *Campylobacter enteritis* have been steadily increasing in almost all countries (Abdussalem and Kaferstein,1994).

Scanty information from developing countries indicates that some of the foregoing changes have been taking place there also (Abdussalem and Kaferstein,1994). In spite of the advances of modern technology, illness due to contaminated food is a leading cause of sickness and death in the developing world, and affect untold millions in all countries, since such diseases are very much under reported (WHO, 1991). These increased situation of the diseases could be due to the deteriorating sanitary conditions in the rapidly expanding towns and cities, where very large numbers of poor and destitute persons have been arriving from rural areas (Abdussalem and Kaferstein,1994).

In spite of the common occurrence of diarrhoea and other food borne diseases, the extent of their real impact on community health remains unknown because only small proportion of cases come to the notice of health services and ever fewer are investigated. Reliable data estimates are not even available from most developing countries. In developed countries, about 1-10% of actual cases may be reported. Thus the attention given to food safety to combat the diseases is quite small. Only dramatic episodes such as out-breaks of cholera, typhoid fever or food poisoning have been given more attention (WHO, 1984). Globally *E. coli* alone can contribute for about 25% of the diarrheal episodes in infants (Motarjemi *et al.*,1993,1994). The enterotoxigenic *E. coli* annually claims the lives of 800,000 children under age of five and causes diarrhea on about 650 million of them (Gaastra and Svennerholm, 1996). Shigellosis is a major health problem in developing countries and causes 10-15% of acute diarrhea in children (Motarjemi *et al.*,1993). Since 1960 *Shigella dysenteriae*

type 1 epidemic and endemic dysentery in various parts of the world, in America, Mexico, Central Africa and Indian sub continent resulted in an estimated one million cases and death of thousands (Strockbin *et al.*,1991).

Staphylococcus and *Salmonella* food borne diseases are the other serious problems. They are estimated to be the cause for over 43% of the total out break and over 67% of the illness in USA (Banwart, 1979). The number of outbreaks and sporadic cases of human salmonellosis is steadily increasing in many countries (Ruz *et al.*1987), with about 4.1% mortality rate (Jay, 1992).

Although the majority of the above mentioned food-borne diseases are self limiting, they can also be life-threatening that need antibiotic therapy. But most of them have already developed resistance to common antibiotics in many countries *E. coli* (Pinger and Cooke, 1985), *Salmonella* (Salyers and Whitt, 1994; Jawetz, 1976) and *Shigella* (Nikkah and Mehr-Movahel,1988; Alcamo, 1994, Guyot, 1996), *Stapylococcus* (Volk *et al.*,1991) have already developed resistance to commonly used drugs. In general the enterobacteriaceae, in developing countries, have become resistant to commonly used antibiotics (Shears *et al.*, 1988).This type of resistance is also reported in Ethiopia by various authors (Abraham and Girmay, 1997; Messele and Alebachew, 1979; Afeworki and Drassar, 1989; Afeworki and Yetnebersh, 1980; Abebe *et al.*,1997; Mogessie and Messele, 1985; Lindtjorn *et al*, 1989; Girma, 1988).

In most developing countries where many people are living under poor hygienic condition, the chance of contracting food borne disease undoubtedly is high. Even if there are medical centers, due to economic reasons as well as lack of awareness of modern medication, people prefer to visit traditional healers. According to Akerele (1993), 80% of the world population depends on traditional medicine. About 70-80% of the population in Africa do not have access to basic modern health-care services such as protective immunization, assistance of mother during pregnancy and child birth, post-natal and infant care, health and nutritional education, safe water supplies and first aid/emergency case (WHO, 1996). Thus they depend on traditional medicine to treat themselves. In Zimbabwe for example 80% of the population depends on traditional medicine as life saving drugs are beyond their reach (Nyazema and Kire, 1986). In Ethiopia, too, 80% of the population also depends on traditional medicine (Dawit, 1996).

Traditional medicine is defined as the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, either verbally or in writing (WHO, 1976).

Some traditional healers use medicinal plants. Some of the medicinal plants, although they are not investigated scientifically, can cure certain infections (Andrews, 1982). It can also be assumed that the major part of traditional therapy involves the use of plant extracts or their active principles (Akerele, 1993). If proper investigation is made,

they can serve as a source of modern drug. In fact, in the past also, they have been the source of modern medicine (Bakhiet and Adam, 1995) . Drugs such as quinine, digitalis etc. have been synthesized from medicinal plants (Mesfin, 1986). They also served as a source of intermediate for synthesizing analog drugs with more desirable properties (Andrews, 1982). In developing countries their use has helped to substitute imports of drugs, thus boosting economic self-reliance. Furthermore, local products tend to be more readily accepted than those obtained from abroad (Akerele, 1993).

In the past, development of new antibiotics has been one solution for drug resistance pathogens. But today the lack of new or alternative antimicrobial agents for drug-resistance pathogens is making serious out-breaks possible (Cohen, 1992). Vaccine trials have been made on cholera and shigellosis. However, these efforts are still at the research or experimental stage (Motarjemi et al.,1993). Thus one has to look for alternative ways to fight these pathogens. One way is searching for new drugs. But so far only 7% of those presently known to science have been investigated properly. Thus research in traditional medicinal plants might bring new, superior or cost-effective drugs, which might be employed in the fight against several diseases including those that remain intractable or which may newly emerge (Dawit, 1996).

Among African countries Ethiopia is often quoted as one of the six countries where about 60% of the plants are said to be indigenous with a healing potential (Belachew, 1984; cited in Mirgessa, 1996). There are about 213 families of flowering plants in Ethiopia and of these, 92 families, with one family of gymnosperm and one family of

fern, are known to contain species with medicinal properties (Sue Edwards and Zemedede, 1992).

As has been indicated, a large (about three fourth) of the population of the world uses traditional medicine as a means to alleviate his illness. The use of medicinal plants, however, has its own problems. Most of them, if taken in large doses, produce stronger physiological reactions and may have effects greater than the effect of the disease to which they are used against (Andrews, 1982). Their dosage is not precisely known or if it is known through experience, it is vague (Sofowora, 1982) and the constituent of the active ingredients may vary from plant to plant and even within the same plant (Randor *et al.*,1994). Despite their short coming, the use of water extracts from medicinal plants in curing diseases is increasing instead of the synthetic ones. However, when these crude extracts are administered, their cytological effects must be serieously studied (Adam and Farah, 1989) and using extracts for all types of diseases without considering specificity should be avoided.

In spite of traditional importance of medicinal plants, only few studies have been done in Ethiopia (Mirgessa, 1996). The purpose of this study is, therefore, to evaluate the antimicrobial potential of some of the medicinal plants *Artemisia afra*, Ariti ; *Vernonia amygdalina*, girawa ; *Lepidium sativum*, Feto ; *Carum copticum*, Nech Azmud ; *Zingiber officinale*, Zingibel ; *Echinops* species, 'Kebericho ; *Coriandrum sativum*, Dembelal ; *Cymbopogon citratus*, Tej sar ; *Foeniculum vulgare*, Enselal ; *Ruta chalepensis*, tena Adam and *Syzygium guineense*, Dokema against *Staphylococcus aureus*, *Bacillus cereus*, *Shigella flexneri*, *Shigella boydii*, *Salmonella typhimurium* and *Escherichia coli*. In addition, the minimum inhibitory concentration, the minimum bactericidal concentration and the bactericidal activity of the crude preparations will be determined. These plants are recorded in the literature as being used by traditional healers to treat diarrhoeal diseases (Amare, undated: Kloos et al., 1987; Sue Edwards and Zemedu, 1992.)

□

2. LITERATURE REVIEW

2.1. General Characteristics of the pathogens

2.1.1. *Bacillus cereus*

Members of the genus *Bacillus* belong to the family Bacillaceae and are characterised by their rod shaped morphology, appearing sometime in chains, and ability to produce endospores. They are motile by means of peritrichous flagella or non-motile. Majority of the members are Gram-positive but some are Gram-variable or negative. Most species of *Bacillus* are not fastidious. They grow well in aqueous extracts of soil, in vegetable or yeast extracts, or in simple peptone media (Frobisher, 1968). The optimum T^o for their growth is 30^oc, with minimum T^o for growth at 10^oc and maximum 49^oc. The pH range for growth is 4.9 and 9.3 (Frasier and Westhoff, 1978). All the members are more or less strictly aerobic (Frobisher, 1968).

Bacillus cereus is widely distributed in nature and has been found in air, soil, water, milk, dust, cocoa powder, meat brines, semi-preserved meats, spices, plant products (cereals, flour, starch, bakery products), mixtures of ingredients (spaghetti sauce, pudding, soup mixes, gravy mixes). It is nearly isolated from all plant foods and dehydrated foods. But it is unable to grow on foods with pH values below and above 4.7 and 9.5, respectively (Chung and Sun, 1986; Troller; 1976; Banwart, 1979; Portnoy *et al*, 1976).

Bacillus cereus is known to produce two types of toxins, the diarrhoeogenic (heat-labile) and the emetic (heat-stable) toxins, causing diarrhea and vomiting, respectively (Terranova and Blake, 1978). The emetic toxins (Melling *et al*, 1976; Volk *et al.*, 1991) are only produced when the organism grows in rice. Production of emetic toxin has not

been observed when it grows in other food. Unlike the emetic, the diarrhoeal toxin can be produced by the organism in any food item (Melling *et al*, 1976; Turnbull, 1976). The enterotoxin is believed to be produced during the logarithmic phase and is released up on lysis of the cells (Banwart, 1979). A large inoculum ($>10^6$) or about 10^8 per gram of viable cells of *B. cereus* must be ingested to develop the signs and symptoms. The symptoms include nausea, abdominal cramps, watery diarrhea and vomiting. The duration is short about one day or less (Fraser and Westhoff, 1978).

2.1.2. *Escherichia coli*

The genus *Escherichia* is composed of motile or non motile bacteria that conform to the definition of the family Enterobacteriaceae and the tribe Eschericheae (Ewing, 1967). Among the species of the genus *Escherichia*, *E. coli*, is a motile Gram-negative rod without capsules or spores. The genus in general is known by its ability to produce both acids and gas from a wide variety of fermentable carbohydrates.

Escherichia coli consists of a complex O, K and H antigenic structures. Comparison of these antigens (especially O and K antigens) by serological method has for classifying strains of *E.coli* into a number of serotypes (Buxton and Fraser, 1977).

Escherichia coli is aerobic and facultatively anaerobic. It is readily grown on ordinary laboratory media without the addition of blood, serum fluid, glucose etc. The optimum temperature for cultivation is 37°C but growth will occur over a temperature range of approximately 20-44°C (Buxton and Fraser, 1977). Although rapid growth occurs at 27°C, prolific growth also occurred within 24 h when cultures were held at 30 to 40°C. Growth

was shown above 44 to 45°C. But *E. coli* 0157: H7 could survive in ground beef at -20°C for several months without major change in numbers (Doyle and Schoeni, 1984). The optimum pH for growth is 7 to 7.5 with the minimum at pH 4 and maximum at pH 8.5 (Frasier and Westhoff, 1978).

Escherichia coli is a normal inhabitant of the intestinal tracts of vertebrates including man. The organism has been isolated from various domestic animals. The enteropathogenic *E. coli* (EPEC) and the enteroinvasive *E. coli* (EIEC) diarrhea occurs with a world wide distribution. A majority of cases have been reported in industrialised urban parts of the world (Dupnot, 1982). Enterotoxigenic *E. coli* has been reported from various corners of the globe. The various groups the EIEC, EPEC, ETEC have different mode of pathogenesis. The EIEC invades and proliferates within epithelial cells and eventually cause death of the cell (Law, 1994, Tziouri et al., 1989).

Escherichia coli has been resistant to many antibiotics. Sensitive strains of the organism are known to accept transferable resistance factor from other organisms like *Salmonella* (Gill and Hook, 1966; Multugh, 1975). There are reported cases of resistance of *E. coli* to a wide variety of antibiotics. Abraham and Girmay (1997) in Gondar have isolated *E. coli* resistant to tetracycline, chloramphenicol, co-trimoxazole, ampicillin and gentamicin.

2.1.3. *Salmonella*

The genus *Salmonella* in general is composed of motile bacteria that conform to the definition of the family Enterobacteriaceae and the tribe Salmonellae. At present there are more than 2000 serotypes of *Salmonella* and each year quite a few are isolated,

characterised and added to the list (Saxena et al. 1980). The classification of *Salmonella* by serotyping is based on the original work of Kauffmann and White and is often referred as the Kauffmann-white scheme.

Generally members of the genus *Salmonella* are not nutritionally fastidious. They are facultative aerobes and grow well when incubated under normal aerobic conditions (Troller, 1976). The pH range is from 4.1 to 9. The lowest water activity for growth varies with the food but is about 0.93 to 0.95 (Frasier and Westhoff, 1978).

Although man serves as the main reservoir of infection, animals especially those whose flesh serves as food, such as turkeys and chickens or those present in the home as dog and pet turtles, also play a role in the spread of salmonellosis (Tartakov and Vorperian, 1981). Reptiles and Rodents also harbour the agent (Volk et al, 1991; Burrows, 1969). Generally a variety of food have been incriminated for an outbreak of salmonellosis. The most commonly incriminated are various kinds of meats, poultry and products from them (Frasier and Westhoff, 1978).

Although there are few reports due to the self limiting nature of the disease, the actual rate of infection due to *Salmonella* is expected to be large. Most morbidity cases are expected to be unreported. In Ethiopia, *Salmonella* was isolated from various sources and patients (Sahlu, 1983; Mogessie and Messele, 1985; Mogessie, 1994; Mezgebe and Mogessie, 1998; Wolde-Aregay and Mogessie, 1998)

The pathogenesis of *Salmonella* always involves the ingestion of viable organisms. In adult healthy male individuals to elicit diarrhea about 10^5 viable cells are required

(Dupnot and Hornick, 1973 Cited in Mogessie, 1983). But it has been shown that few number of *Salmonella* also can cause the disease (D'Aoust and Pivnick, 1976). The pathogenesis of *Salmonella* involves two toxins, the enterotoxin and cytotoxin (Jay, 1992).

Resistance to antimicrobial agents has become a significant issue. Isolates obtained both from animal and man have been found resistant to several antimicrobial agents and much of this resistance is plasmid mediated (Anderson, 1968).

The organism have already developed resistant to many antimicrobial agents and many of them are transferable. In Ethiopia resistant *Salmonella* have been reported (Messele and Alebachew, 1979; Lindtjorn *et. al.*, 1989; Mogessie and Messele, 1985).

2.1.4. *Shigella*

The genus *Shigella* is composed of Gram-negative non-motile bacilli. With the exception of a certain biotypes of *Shigella flexneri* 6, visible gas is not formed from fermentable carbohydrates. Compared with *Escherichia*, *Shigella* are less active in their utilization of carbohydrates. Biochemically the genus is divided into groups based on their ability to ferment mannitol. The non-mannitol fermenting group, is designated as Group A (*Shigella dysenteriae*) and the mannitol fermenting as group B (*Shigella flexneri*), group C (*Shigella boydii*) and group D (*Shigella sonnei*). Infact the basis for classifying the mannitol fermenting group in to various groups is their antigenic basis.

The nutritional requirement of *Shigella* is not complex. Thus they can grow on ordinary nutrient (beef extract) media. Like most other intestinal diseases, shigellosis is spread by faecal-oral transmission by man or by domestic animals harbouring the bacilli in their faeces and by contaminated food or water. Contaminated inanimate objects also can help the dissemination of the causative agent. The agent can also be transmitted by flies as far as the agent is in their faeces (Tartakow and Vorperian, 1981). Moist mixed foods such as milk, beans, potato, tuna, shrimp, turkey, macaroni salads and apple cider have been implicated as a vehicle food (Frasier and Westhoff, 1978; Hayes, 1995). The disease can be spread by person-to-person contact (Salyers and Whitt, 1994).

Shigellosis is no longer a major public health problem in developed countries, except in a few high-risk groups, due to improvement in housing, personal and environmental sanitation, and nutrition. But in developing countries it is still a serious problem causing a great deal of morbidity and mortality particularly in infants. In Ethiopia Afeworki and Yetnebersh (1980) reported the isolation of 165 *Shigella* strains between the year 1974 and 1978. Messele and Alebachew (1982) also have reported the isolation of 105 *Shigella* strains between the year 1975-1980. Mogessie and Messele (1985) also have isolated *Shigella* from 9% of adult out patients among 1000 diarrheal cases. As *Shigella* is able to manage to pass conditions in the stomach, most ingested organisms can reach the intestine alive, therefore only few about 100 to 200 bacteria can serve as ID₅₀ for humans.

The incubation period of the uncomplicated case ranges from 1-7 days usually less than 4 days. Symptoms of the disease includes abdominal cramps, fever, chills, diarrhea, watery

stools frequently containing blood, mucus or pus's, tenesmus, headache, lassitude, prostration, nausea and dehydration (Frasier and Westhoff, 1978).

Shigella is the first organism from which plasmid-mediated multiple drug resistance has been noted (Watanabe, 1963). This led to a spectacular rise in the prevalence of high drug-resistant *Shigella* in Japan and elsewhere in the world as antibiotic pressure led to selection of the plasmid-bearing organisms. Resistant strains of *Shigella* have been isolated from various parts of the world. In Ethiopia various workers have investigated the existence of resistance to one or more drugs among *Shigella* isolates (Mogessie and Messele, 1985, Afeworki and Drassar, 1986; Afeworki and Yetnebersh, 1989; Abebe et al., 1997; Messele and Alebachew, 1979). Most strains elsewhere have developed resistance to commonly used drugs (Nikkah and Mehr-Movahel, 1988; Alcamo, 1994; Guyot, 1996 and Lindtjorn et al, 1989).

2.1.5. *Staphylococcus aureus*

Staphylococci occur singly, in pairs, in tetrads and in irregular clusters, especially when grown in broth. They are Gram-positive and are members of the family Micrococcaceae (Evans, 1956). The organism grows well anaerobically in the presence of fermentable carbohydrates but grow even better aerobically. It grows at 10°C and 45°C, the optimum being 37°C (Evans, 1956). The pH optimum at 37°C is 7.5 and the organism is highly tolerant to high salt concentration such as 8% (Iandolo and Schafer, 1977). However growth and enterotoxin production were better when pH and salt concentration's were decreased (Genigeorgis and Sadler, 1966). It also tolerates dissolved sugar up to 50 to

60% (Frasier and Westhoff, 1978). The organism is quite distinct from other food borne pathogens by its ability to grow at water activity, (a_w), below 0.9 (Bae and Miller, 1992).

Foods involved in staphylococcal food poisoning are most often starch containing materials such as custards used in cakes, and éclairs, gravies, dressing salads etc. (Burrows, 1969) pastries, chapped and sliced meats, ham and bacon are also implicated. Food may be contaminated with pus from food handlers infected finger or abscess on an exposed pus, or by nasal secretion. Even the organism may be found on hands with normal skin. Contaminated milk from a cow with mastitis, as well as milk products such as cream, cheese may also serve as sources of infection. (Tartakow and Voreperian, 1981). In Ethiopia Mogessie and Yewelsew (1996) have isolated a high number $>1 \times 10^5$ cfu/g of *Staphylococcus aureus* from Bulla and Kotcho; Generally *Staphylococcus* is expected to exist, at least in low numbers, in any or all food products that are of animal origin or in those that are handled directly by humans unless sufficiently heat treated to effect destruction of the organism (Jay, 1992). Diseases due to *Staphylococcus aureus* has a world wide distribution (Finland, 1973).

Members of the genus *Staphylococcus* in general cause a myriad of infections ranging from localized furuncles and carbuncles to food poisoning, pneumonia, meningitis and disseminated infections involving any organ of the body (Volk et al., 1991). They produce a variety of enzymes and toxins to invade any tissue and organ in the body. They produce hemolytic toxins, leucocidin, hyaluronidase, coagulase, fibrinolysin, deoxyribonuclease, phosphatase, lysozyme and enterotoxin.

In staphylococcal food-poisoning (gastronenteritis) syndrome, the organism elaborates seven different enterotoxins designated as (staphylococcal enterotoxin (SE) A, B, C (C₁,C₂ & C₃), D & E. They are simple proteins. Among the enterotoxins SEA is the serotype most commonly associated with food borne diseases (Salyers and Whitt, 1994). Pathogenicity usually is associated with coagulase and thermostable nuclease production. Those strains producing coagulase and thermostable nuclease usually are considered as pathogenic, but all pathogenic staphylococci are not coagulase and thermostable nuclease producers (Mitruka, 1976).

The incubation period after feeding the contaminated food may be as short as 2 hours, but in general, it is 4 to 6 hours (Hobbs & Gilbert, 1978). The symptom includes salivation, nausea, vomiting, abdominal pain, head ache, sweating, chills may occur. The duration is brief one day or two (Frasier and Westhoff, 1978).

Staphylococcus aureus appear to be consistently adapted in becoming resistant to drug therapy. As a result many antibiotics soon become ineffective for treatment of staphylococcal infection. In Ethiopia various workers have found resistant strains to various antibiotics (Abraham and Girmay, 1997; Wezenet, 1982; Lindtjorn et al., 1989; Girma, 1988).

2.2. Description of the Medicinal Plants

2.2.1. *Artemisia afra* Jacq.

Artemisia afra is a member of the family compositae (Hedberg and Sue Edwards, 1995).

It is a herbaceous shrub reaching about 1m high.

2.2.2. *Carum copiticum* Hiern.

Is a decumbent or erect annual herb, up to 160 cm high, with a light-yellow taproot and many side roots; all green parts with a broom (Janseh, 1981). It is member of the family Umbelliferae (Shukla and Mistra, 1994).

2.2.3. *Coriandrum sativum* Linn.

Coriandrum sativum is the member of the family umbilliferae. It is an annual plant about 0.7m high with white or pinkish flowers, with erect, slender, smooth stem, branched in the upper part. It is indigenous to Italy, but widely cultivated in Holland, centrel and eastern Europe the Mediterranean (Morocco, Malta, Egypt), China and India. It is also cultivated in south and central America and USA(Trease, 1978; Yongken 1950).

2.2.4. *Cymbopogon citratus* (DC.) Stapf.

Cymbopogon citratus is a perennial densely tufted cultivated grass (Kotb, 1985). Often tall and robust. A member of the family Poaceae (Gramineae. Essential oils is present in all *Cymbopogon* species and the aromatic flavor of the leaves when chewed is useful to distinguish the genus (Hedberg and Sue Edwards, 1995).

2.2.5. *Echinops* spp.

Members of the genus belongs to the family Compositae. They are herbs, rarely arborescent, with generally alternate, more rarely opposite, extipulat leaves (Rendle, 1979). Leaves and stem spiny, thistle-like, flower head spherical (Polunin and Huxley, 1965).

2.2.6. *Foeniculum vulgare* Mill.

Foeniculum vulgare is member of the Umbelliferae. It is a perennial aromatic herbs attaining a height of about 1m with a green, furrowed, branched stem bearing alternate, twice pinnate leaves with narrow pinnae (Yongken, 1950; Heywood, 1993).

2.2.7. *Lepidium sativum* Linn.

Lepidium sativum is the member of the family Cruciferae. It is an erect annual herb about 20-80cm high (Janseh, 1981; Rendle, 1979).

2.2.8. *Ruta chalepensis* Linn.

Ruta chalaepensis is indigenous to the Mediterranean area, the Canary islands, Arabia and Somalia. It is cultivated all over the world in many countries. The plant is an erect perennial herb becoming woody at the base, and up to 1.5 m high (Janseh, 1981).

2.2.9. *Syzygium guineense* (willd) DC.

It is a tree with tall stem and is the member of the family Myrtaceae with a juicy black, when ripe, edible fruits. The wood is reddish-white turning darker red, hard, fairly strong and very durable. The bark, roots and leaves are astringent and are used for diarrhoea and in prescriptions for venereal discharges (Hutchinson and Dalziel, 1954).

2.2.10. *Vernonia amygdalina* Del.

Vernonia amygdalina is a woody shrub, growing in sub-humid wooded savannah or wetter highlands in dry moist and wet Weyna dega' and Dega' agronomic zone of 1,000-2,700m. It is a single-stemmed shrub to 3m high, sometimes a tree to 10m (Azene *et al*, 1993).

2.2.11. *Zingiber officinale* Roscoe.

Is the member of the family Zengiberacea. The plant is a perennial herb, with a subterranean, digitately branched rhizome that sends up stems that are covered by leaf sheaths. It is extensively cultivated in tropical localities (Yongken, 1950). It grows well in subtropical areas where the rainfall is at least 80 in per annum and grows in many parts of the world including Jamaica, China, India and Africa (Trease, 1978).

□

3. MATERIALS AND METHODS

3.1. *Sample Collection*

Eleven various types of Ethiopian traditional medicinally used plants were either collected or purchased from local markets. The seeds of *Carum copiticum*, *Coriandrum sativum* and *Lepidium sativum*; the leaves of *Ruta chalaepensis*, *Cymbopogon citratus* and *Artemisia afra* the roots of *Echinops* spp and the rhizome of *Zingiber officinale* were purchased from local markets (Merkato, Sholla and Aware). The leaves of *Vernonia amygdalina*, *Syzygium guineense* and *Founiculum vulgar* were collected from home as well as Addis Ababa University science faculty gardens.

3.2. *Preparation*

The appropriate parts of the plant were cleaned with sterile distilled water. The seeds, the root and the rhizome were washed while the leaves were cleaned by soaking in sterile distilled water. The cleaned plant parts were then sun dried, powdered and sieved with a mesh.

3.3. *Sample Treatment*

3.3.1. *Sterilization*

Known mass of the prepared powder of each medicinal plant was dissolved in distilled water to give a concentration as high as possible to be pipetted with a sterile Pasteur

pipette. This was sterilized at 121°C for 15 min. The concentration of the various plant materials prepared was as follows:- *Carum copticum* and *Coriandrum sativum*, 20%; *Ruta chalaepensis*, *Foiniculum vulgare*, *Cymbopogon citratus*, *Echinops spp*, *Vernonia amygdalina*, *Syzygium guineense*, and *Zingiber officinale* 10%; *Artemisia afra*, and *Lepidium sativum*, 5% (weight/volume).

3.3.2. Tyndalization

Tyndalization of various concentrations of the plant materials was made in an incubator with steam. The temperature of the incubator was adjusted to 100°C and after that the samples were heated for exactly 30 minutes and left for about 24 h at ambient temperature and reheated again in the same incubator at the same temperature for exactly 30 minutes. The same procedure was repeated again after keeping the sample at ambient temperature for another 24 h.

3.3.3. Boiling for 10 Minutes

The crude preparations of the various medicinal plant concentrations were boiled in a beaker partially filled with water, on an electric stove at 95°C for 10 minutes.

3.3.4. Heating at 80°C for 10 minutes

The crude preparation of the various medicinal plant concentrations were heated at 80°C for 10 minutes in a water bath.

3.3.5. Pasteurization

Pasteurization of the crude preparations was made by heating the preparation at about 62.8°C for 30 minutes in a water bath.

3.3.6. Lyophilization

Known mass of the powdered plant material to fit the same concentration, as used in 3.3.1., was soaked in 70% ethanol for 24 h and then dried in a rotavapour at 40°C. After all the ethanol was removed, the remaining preparations were lyophilized after deep freezing the sample at -50°C until it became firmly solid.

3.3.7. pH treatment

The crude preparations of *Ruta chalaepensis* and *Echinops spp.*, were pH treated at a fixed concentration, 10%. The pH adjustment was made with 0.1 M NaOH and HCl to give final pH values of 5, 6, 7, 8 and 9. The adjustment was made carefully not to add an excess of either of these solutions to the crude preparations. Then the preparations were pasteurized as indicated above. These plants were selected in this test because they showed inhibitory activity in agar diffusion assay.

3.4. Screening for Antimicrobial Activity

3.4.1. Standardization of Inoculum

For antimicrobial testing the following identified bacteria were kindly supplied by Dr. Abera Geyid (EHNRI): *Staphylococcus aureus* (ATCC 29213), *Bacillus cereus* (isolate), *Shigella flexneri*, (isolate) *Shigella boydii* (isolate) *Escherichia coli*. (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028).

A loopful (1-2) of the test bacterial strains was separately inoculated into a sterile Muller Hinton broth. After incubation for 24h at 37°C the cultures were compared with 0.5 McFarland turbidity standard made by adding 0.5ml of 0.048M BaCl₂ to 99.5ml of 0.35N H₂SO₄ following standard procedures (Thrupp, 1980). Each time before use the preparation was agitated on a vortex mixer. Cultures, if found turbid than the standard, were serially diluted to get the required concentration.

For *B. cereus* the 0.5 McFarland turbidity standards did not work as it attained about 10⁷ cfu/ml after 24 h. Instead a 24 h culture of the organism was used to inoculate the material after serial dilution.

After adjusting cultures with 0.5 McFarland turbidity standard, prior to inoculation, they were diluted with sterile distilled water to achieve a final concentration about 10⁶ cfu/ml.

3.5. Determination of Minimum Inhibitory Concentration (MIC)

3.5.1. The Agar Well Plate Diffusion Assay

The agar well plate diffusion assay was carried according to Barry (1980) with slight modification. For this assay sterilized Muller Hinton agar was poured into pre-sterilized 9cm Petri dish and was left to dry. On the dried Muller-Hinton agar 2ml of a standardized and diluted 18-24h broth culture, grown in Muller Hinton broth was added on to the surface, distributed thoroughly by rocking and was left to dry for 15 minutes at room temperature after removing the excess inoculated culture. On to this inoculated media six pores were aseptically made, each 6mm in diameter, using the bottom end of an inverted sterile 1 ml pipette.

To five of the wells, 2-3 drops of the various concentrations of the crude preparation of the plant materials were added using a sterile dropper. The last well was filled with sterile distilled water as a control. The plates were then incubated for 24h at 37^oc. The diameter of the clear zone was measured using a transparent ruler from the bottom of the petridish. The MIC was the minimum concentration that gave a clear zone around the well. Each of these experiments was conducted in triplicates.

3.5.2. Agar Dilution Assay

The antimicrobial activity as well as MIC of the crude preparation was also determined by agar dilution streak method, with modification of the method used by Mitscher *et al.* (1972). Using this method three sets of tests were carried out.

3.5.2.1. Assay by pasteurized Preparation

To prepare a pasteurized plant material the powder of the various medicinal plants were dissolved in sterile distilled water, pasteurized and mixed with a sterile molten Muller Hinton agar at about 50°C. The concentration of the powder-water mixture and the Muller Hinton agar was carefully adjusted to fit the required concentration and to allow the media to solidify. Then the Agar was poured into pre-sterilized Petri dish. To the solidified medium a loop full of the standardized culture of each organism was streaked and incubated for 24h at 37°C and observed for growth.

3.5.2.2. Assay by Sterilized Preparation

The powder of the various medicinal plants was processed as in 3.5.2.1. and autoclaved. To the solidified media a loopful of the standardized culture of each organism was streaked and incubated for 24h at 37°C and observed for inhibition.

For those able to inhibit the growth of any of the test organisms further work was done to determine the MIC growth. The MIC is the minimum concentration that totally inhibit the growth of the test organism.

3.5.2.3. Assay by Sterilized Preparation of pH 7.

The procedure is the same as in 3.5.2.2 except that the pH of the mixture was adjusted to pH 7 before autoclaving.

For all the above tests control was made using Muller Hinton agar free of the crude preparations and all the tests were conducted in triplicates.

3.5.3. *Determination of Bactericidal Concentration*

Determination of the minimum bactericidal concentration (MBC) was made following Thrupp (1980) with slight modification. Series of doubling concentrations of the plant material, which showed inhibition in 3.5.2.2, were made by dissolving known mass of the powder of the plant material in 9ml Muller Hinton broth and the mixture was sterilized at 121°C for 15 min. A tube containing only Muller Hinton broth served as control. One ml of a standardized culture of the susceptible organism containing about 10^6 cfu/ml were inoculated separately to each broth to get a final inoculum level of about 10^5 cfu/ml. Just at the time of inoculation, sample was drawn from the control tube and 0.1ml of the appropriate dilution of the culture was pour plated on Nutrient Agar to determine the inoculum level. All tubes were incubated at 37°C for 24h. Counting of control and other plates was done after 24 hours. Comparison was made between the control plate and the others to determine the MBC. The MBC was defined as the minimum concentration that kills more than or equal to 99.9% of the inoculated (initial) bacterial population. These test was conducted in duplicates.

3.5.4. *Determination of Bactericidal Activity*

Bactericidal activity was determined using the methods of Thrupp (1980) with slight modification. Known mass of the powder of the various medicinal plants with antibacterial activity were prepared to fit their respective bactericidal concentration by mixing with 9ml Muller Hinton broth. The pH of each mixture was measured using a digital pH meter before sterilization. After sterilization, 1 ml of the standardized susceptible culture containing about 10^6 cfu/ml was inoculated to make a final concentration of about 10^5 cfu/ml. Sample were drawn at 0 h from the control tube and appropriate dilution was pour plated onto Nutrient Agar. Counting was done at 4 hour intervals until 24 h. This experiment was carried out in duplicates.

3.5.5. *Assessing the General Effects of the Sterilized Crude Preparation at a Certain Concentration.*

The effect of sterilized crude preparations of all test plants on all test organisms was assessed using the maximum possible concentration of the crude preparations that could allow pipetting.

4. RESULT

4.1. Agar well plate diffusion assay

4.1.1. *Sterilized and tyndalized crude preparation.*

Of the tested medicinal plants at different concentrations with the above treatments none showed zones of inhibition.

4.1.2. *Crude preparation boiled for 10 minutes*

Of the crude preparations at the tested concentrations only 2 plants , *Ruta chalaepensis* and *Echinops* spp. gave clear zones against *Bacillus cereus* . For these further study were made to determine their minimum inhibitory concentration.

Table 1. Average size of clear zone (in mm) formed by the crude boiled (for 10 minutes) preparation of various concentration of *Ruta chalaepensis* and *Echinops* spp. against *Bacillus cereus*.

Concentration %(Weight/ Volume)	<i>Ruta chalaepensis</i>	<i>Echinops spp.</i>
20	-	12.5
10	8.3	11
5	7.3	10
2.5	6.3	7.5
1.25	0	0

The minimum inhibitory concentration (MIC) for both *Ruta chalaepensis* and *Echinops* spp. was 2.5% (Table 1). The correlation coefficient between the diameter of the clear zones formed and concentration for *Ruta chalaepensis* was 0.7507(p=0.249) and it was 0.7380(p=0.155) for *Echinops* spp.

4.1.3. Crude preparation heated at 80 °C

The result obtained with this treatment regarding MIC is the same as the above treatment for both preparations i.e their MIC is 2.5%. However the correlation coefficient between the diameter of the clear zone and concentration for *Ruta chalaepensis* is .7385(p=.261) and it is .1385 for *Echinops* spp. with p=.185.

Table 2. Average size of clear zone (in mm) formed by the crude heat treated (at 80 °C for 10 minutes)preparations of *Ruta chalaepensis* and *Echinops* spp. at various conc.'s against *Bacillus cereus*.

Concentration %(W/ V)	<i>Ruta chalaepensis</i>	<i>Echinops</i> spp.
20	-	11
10	9	10
5	8	9
2.5	7	7.5
1.25	0	0

4.1.4. After pasteurization of the crude preparation

The MIC for *Ruta chalaepensis* was found to be 2.5% while for *Echinops* it was lower, i.e 0.625%. The correlation coefficient between the concentration and the diameter of the

clear zone was 0.7385 (p=0.261) for *Ruta chalaepensis* and 0.6504 (p=0.114) for *Echinops* spp.

Table 3. Average size of clear zone (in mm) formed by the crude pasteurized (at about 62.8 ° c for 30 minutes) preparations of *Ruta chalaepensis* and *Echinops* spp. at various conc.'s against *Bacillus cereus*

Concentration %(W/ V)	<i>Ruta chalaepensis</i>	<i>Echinops</i> spp.
20	-	11.3
10	9	10
5	8	9
2.5	7	8.3
1.25	0	8
0.625	0	7
0.312	0	0

4.1.5. Lyophilization of the crude preparation

The MIC of *Ruta chalaepensis* is 5% while it is .625% for *Echinops* spp. The correlation coefficient between the various con.'s and diameter of the clear zones is .9320(p=.068)for *Ruta chalaepensis* and .7308 (p=.062) for *Echinops* spp.

Table 4. Average size of clear zone (in mm) formed by the lyophilized crude preparations of *Ruta chalaepensis* and *Echinops* spp. at various conc.'s against *Bacillus cereus*.

Concentration %(W/ V)	<i>Ruta chalaepensis</i>	<i>Echinops</i> spp.
20	-	12.3
10	12.6	11
5	7.6	9.3
2.5	6	8
1.25	0	7.3
0.625	0	7
0.312	0	0

4.1.6. Effect of pH variation at some fixed concentration

Both crude preparations were effective at pH 7. Slight increase or decrease in pH resulted in decreased inhibition. But inhibitions increased at pH 5 and 9 (Table 5).

Table 5. Average size of clear zone (in mm) formed at fixed conc. of *Ruta chalaepensis* and *Echinops* spp. at different pH against *Bacillus cereus*.

PH	5	6	7	8	9
<i>Ruta chalaepensis</i>	12.6	9.3	10.6	9.6	11
<i>Echinops</i> spp.	12.3	9.6	11.6	9.3	9.6

4. 2. Agar dilution Assay

4.2.1. Pasteurized plant preparation

The result obtained after pasteurization of the various conc. of the crude plant preparation indicated that among the tested 11 crude preparations 8 inhibited *Bacillus cereus*, 3 inhibited *Staphylococcus aureus*, 1 inhibited *Shigella flexneri*, and *Shigella boydii*. While 8 of the tested crude preparations were active on gram +ve bacteria, only 1 was active on gram -ve's (Table 6).

Table 6. Inhibition (+) of bacteria by various pasteurized crude preparations at different concentrations by agar dilution assay.

	Conc. % (w/v)	<i>B. cereus</i>	<i>Staph. aureus</i>	<i>Sh. flex.</i>	<i>Sh. Boydii</i>	<i>E. coli</i>	<i>Salm. typh.</i>
<i>Carum copiticum</i>	10	+	-	-	-	-	-
	5	+	-	-	-	-	-
<i>Coriandrum sativum</i>	10	-	-	-	-	-	-
	5	-	-	-	-	-	-
<i>Ruta chalaepensis</i>	10	+	-	-	-	-	-
	5	+	-	-	-	-	-
<i>Founiculum vulgare</i>	10	-	-	-	-	-	-
	5	-	-	-	-	-	-
<i>Cymbopogon citratus</i>	10	+	-	-	-	-	-
	5	+	-	-	-	-	-
<i>Echinops spp.</i>	10	+	+	-	-	-	-
	5	+	+	-	-	-	-
<i>Vernonia amygdalina</i>	10	+	+	-	-	-	-
	5	+	+	-	-	-	-
<i>Syzygium guineense</i>	10	+	+	+	+	-	-
	5	+	+	+	+	-	-
<i>Artemisia afra</i>	5	+	-	-	-	-	-
<i>Zingiber officinale</i>	10	+	-	-	-	-	-
	5	+	-	-	-	-	-
<i>Lepidium sativum</i>	10	-	-	-	-	-	-
	5	-	-	-	-	-	-
Control		-	-	-	-	-	-

4.2.2. Autoclaved plant material

The result of the agar dilution assay after autoclaving of the crude preparation indicated that out of the total crude preparations only 8 inhibited *Bacillus cereus*, 3 inhibited *Staphylococcus aureus*, one inhibited *Shigella flexneri*, and *Shigella boydii*. Of the total crude plant preparations tested, 5 were with antimicrobial activity on Gram +ve's but only 1 on Gram -ve's. The same result was obtained at 10% and 5% after adjusting the pH of the medium containing the medicinal plants to 7 before sterilization (Table 7).

Table 7. Inhibition (+) of bacteria by various sterilized crude preparations at different concentrations by agar dilution assay. Values in bracket indicats pH values for the tested concentrations.

	Conc. %(W/V)	<i>B.</i> <i>cereus</i>	<i>Staph.</i> <i>Aureu</i>	<i>Sh.</i> <i>flex.</i>	<i>Sh.</i> <i>boydii</i>	<i>E.</i> <i>coli.</i>	<i>Salm.</i> <i>typh.</i>
				s			
<i>Carum coriticum</i>	10(7)	+	-	-	-	-	-
	5(7)	+	-	-	-	-	-
	2.5	-	-	-	-	-	-
<i>Coriandrum sativum</i>	10(7)	-	-	-	-	-	-
	5(7)	-	-	-	-	-	-
<i>Ruta chalaepensis</i>	10(7)	+	-	-	-	-	-
	5(7)	+	-	-	-	-	-
	2.5	-	-	-	-	-	-
<i>Founiculum vulgar</i>	10(7)	-	-	-	-	-	-
	5(7)	-	-	-	-	-	-
<i>Cymbopogon citratus</i>	10(7)	+	-	-	-	-	-
	5(7)	-	-	-	-	-	-
<i>Echinops spp.</i>	10(7)	+	+	-	-	-	-
	5(7)	+	+	-	-	-	-
	2.5	+	+	-	-	-	-
	1.25	+	+	-	-	-	-
	0.625	+	+	-	-	-	-
	0.312	-	-	-	-	-	-
	0.156	-	-	-	-	-	-
<i>Vernonia amygdalina</i>	10(7)	+	+	-	-	-	-
	5(7)	+	+	-	-	-	-
	2.5	-	-	-	-	-	-
<i>Syzygium guineense</i>	10(7)	+	+	+	+	-	-
	5(7)	+	+	+	+	-	-
	2.5	+	+	+	+	-	-
	1.25	+	+	+	+	-	-
	.625	+	+	+	+	-	-
	.312	+	+	+	+	-	-
	.156	-	-	-	-	-	-
<i>Artemisia afra</i>	5(7)	+	-	-	-	-	-
	2.5(7)	-	-	-	-	-	-
<i>Zingeber officinale</i>	10(7)	+	-	-	-	-	-
	5(7)	+	-	-	-	-	-
	2.5	-	-	-	-	-	-
<i>Lepidium sativum</i>	10(7)	-	-	-	-	-	-
	5(7)	-	-	-	-	-	-
Control		-	-	-	-	-	-

The minimum inhibitory concentration attended by the different crude preparations against *Bacillus cereus* was as follows: *Cymbopogon citratus*, 10%; *Carium coriticum*, *Ruta chalaepensis*, *Vernonia amygdalina*, *Zingiber officinale* and *Artemisia afra* 5%; *Echinops* spp., and *Syzygium guineense*, 0.625% and 0.312%, respectively.

The MIC for the crude plant preparation against *Staphylococcus aureus* was: *Vernonia amygdalina*, 5%; *Echinops* spp., 0.625%; and *Syzygium guineense*, 0.312%. The MIC against *Shigella flexneri* and *Shigella boydii* for *Syzygium guineense* was 0.3125%.

4.3. Determination of Minimum Bactericidal Concentration (MBC)

Doubling concentrations above the MIC were prepared for those plants that showed activity by agar dilution assay with maximum concentration that permitted pipetting. The result indicated that out of the seven crude preparation that have shown activity by agar dilution assay, only one was bactericidal for *Staphylococcus aureus*, *Shigella flexneri* and *Shigella boydii*. All other were bacteriostatic to *B. cereus* and two of the crude preparations, which showed activity against *Staphylococcus aureus* by agar dilution assay were bacteriostatic. MBC has not been determined for *Artemisia afra* because preparations of liquid media above 7% were difficult to prepare.

Table 8. Results of determination of MBC of the crude preparation of the various medicinal plants against *Bacillus cereus*, *Staphylococcus aureus*, *Shigella flexneri*, and *Shigella boydii*. (All values, unless expressed in the table, are $\times 10^0$).

Bacillus cereus

Conc Weight/vol.	<i>Carium copiticum</i>	<i>Ruta chalepensis</i>	<i>Echinops spp.</i>	<i>Vernonia amygdalina</i>	<i>Syzygium gyneese</i>	<i>Zingiber officinale</i>	Control (Initial inoculum)
20	45		95				
10	85	284	97	128	150	>300	
5	104	>300	113	>300	182	>300	3.65×10^5
2.5			102		193		
1.25			129		>300		

Staphylococcus aureus

Con. weight/vol.	<i>Echinops spp.</i>	<i>Vernonia amygdalina</i>	<i>Syzygium gyneense</i>	Control (Initial inoculum)
20	>300			
10	>300	>300	0	
5	>300	>300	0	2.86×10^5
2.5	>300		3	
1.25	>300		2	
0.625	>300		13	

Shigella flexneri

Conc. weight/vol	<i>Syzygium guineese</i>	Control (initial ioculum)
20		
10	0	
5	0	8.4×10^5
2.5	>300	
1.25	>300	
0.625	>300	

Shigella boydii

Conc. g/ml	<i>Syzygium</i> <i>guineese</i>	Control (initial inoculum)
20		
10	0	
5	0	7.5×10^5
2.5	>300	
1.25	>300	
0.625	>300	

From the above data the MBC of *Syzygium guineese* against *Staphylococcus aureus* was 1.25%. MBC for *Shigella flexeneri* and *Shigella boydii* was 5%.

4.4. Bactericidal activity

The result of the bactericidal activity indicated that all the tested susceptible organisms have been completely inhibited within 8 hours (Fig. 1 and 2)

4.5. General effects of sterilized crude preparation

Of the eleven crude preparations considered in this study, three reduced the final population of *B. cereus* by one log unit, other three by three log units while the remaining three and two reduced the final population by four and five log units, respectively as compared to the control. Among the tested crude preparations eight inhibited the growth of *B. cereus*. Of the crude preparations which showed some degree of inhibition in the previous experiments, three, three and two reduced the final population by one, two and three log units, respectively until the 24 h. Among such crude preparations *Vernonia*

amygdalina was the most effective against *Bacillus cereus*. For *Cymbopogon citratus*, *Syzygium guineense* and *Zigiber officinalis*, reduction in population lasted only until 16 h. The population began to raise thereafter. *Coriandrum sativum*, which did not show inhibitory effect in the other experiments reduced the count of *B. cereus* by 1 log unit after 16 hours of exposure (Fig. 3).

Majority of the crude preparations (10/11) had effect on the final population of *Staphylococcus aureus* at the given concentration. Nine of them reduced the final population by 1 to 3 log unit after 24h and the remaining one by five log units. In fact the latter, *Syzygium guineense* was bactericidal, killing the whole population within 8h (Fig 4).

Unlike the Gram +ves, 10 of the crude preparations did not show any effect on the final population of *Shigella flexneri*. One of them rather enhanced the growth of the organism over the control by one log unit after 24h. The preparation which showed inhibitory property in the previous experiments, however, had bactericidal effect. It reduced the initial population by five log units within 12 hours.

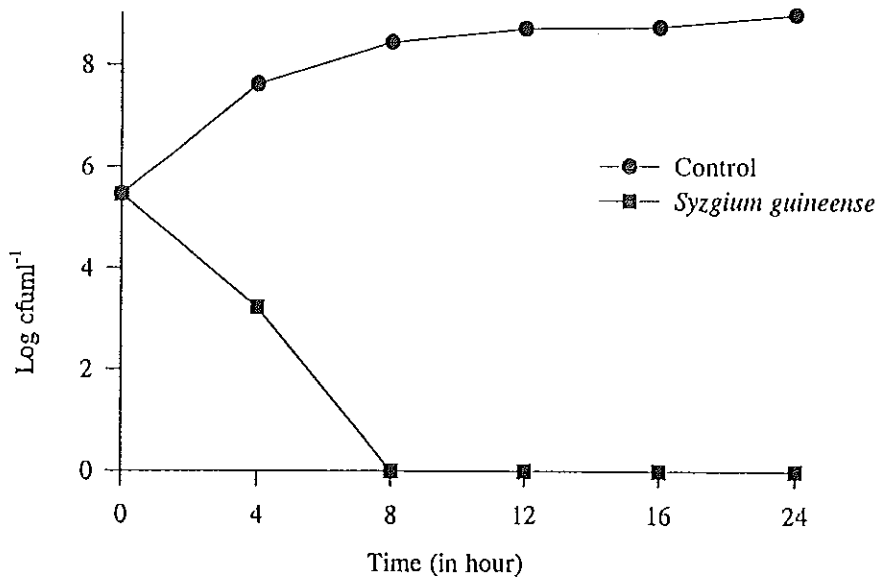


Figure 1. Bactericidal activity of *Syzygium guineense* against *Staphylococcus aureus*. (Con. 1.25% weight/ volume and pH 6.35).

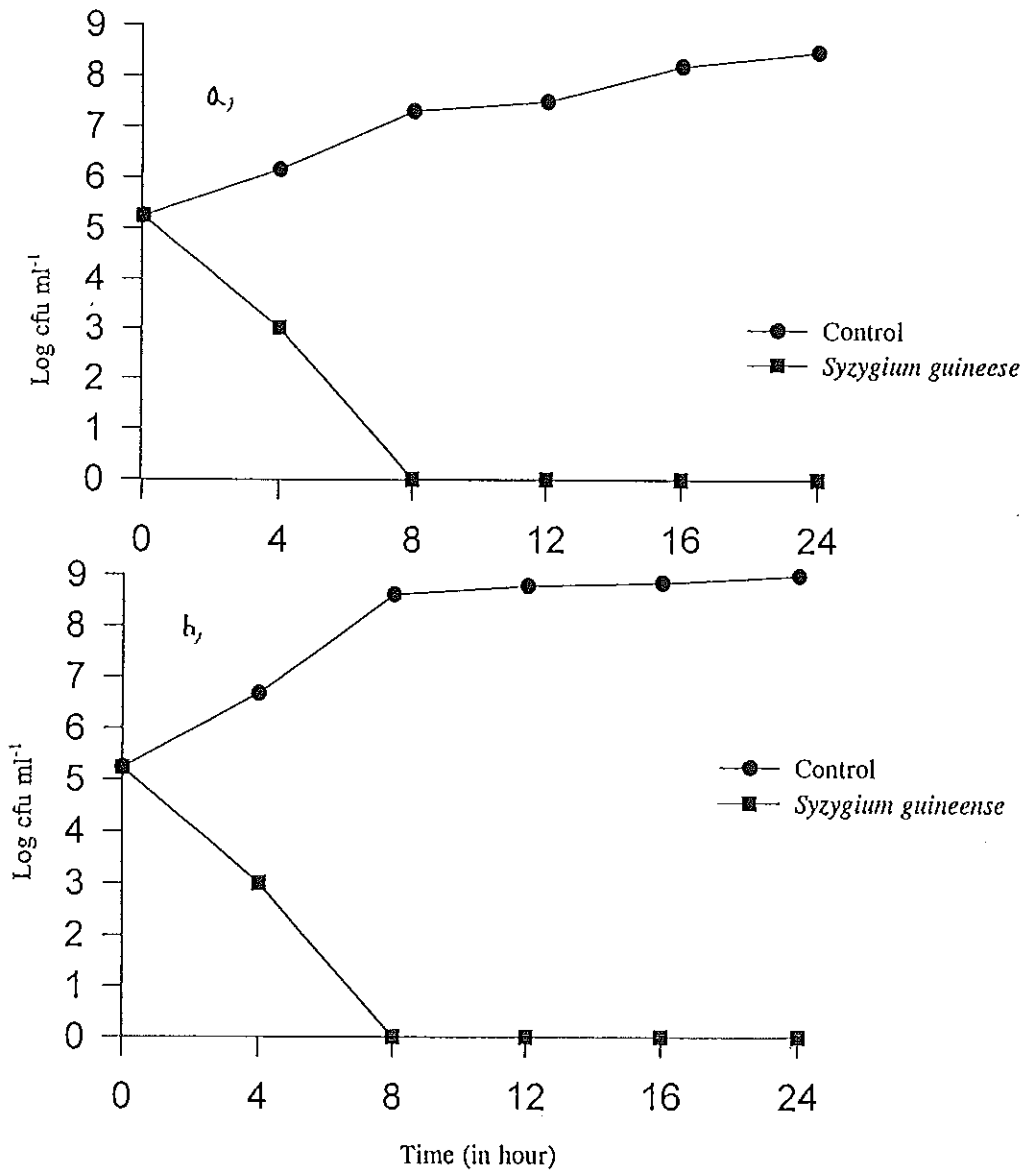


Figure 2. Bactericidal activity of *Syzygium guineense* against a) *Shigella flexneri* and b) *Shigella boydii*. (Con. 5% weight/volume and pH 5.25).

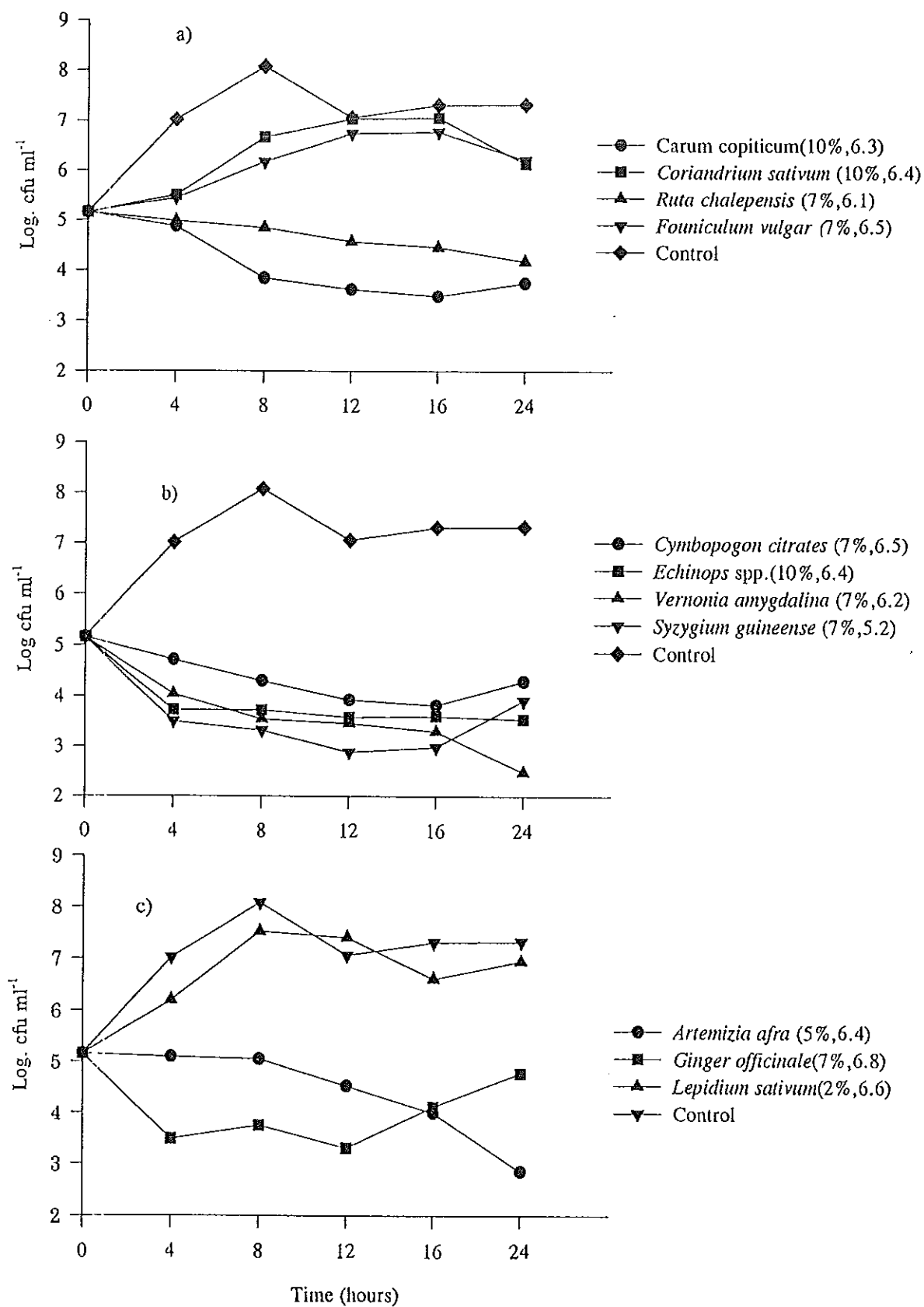


Figure 3 a-c. Response of *Bacillus cereus* to various concentrations of the crude preparations of the medicinal plants. Values in brackets are weight/volume and pH, respectively.

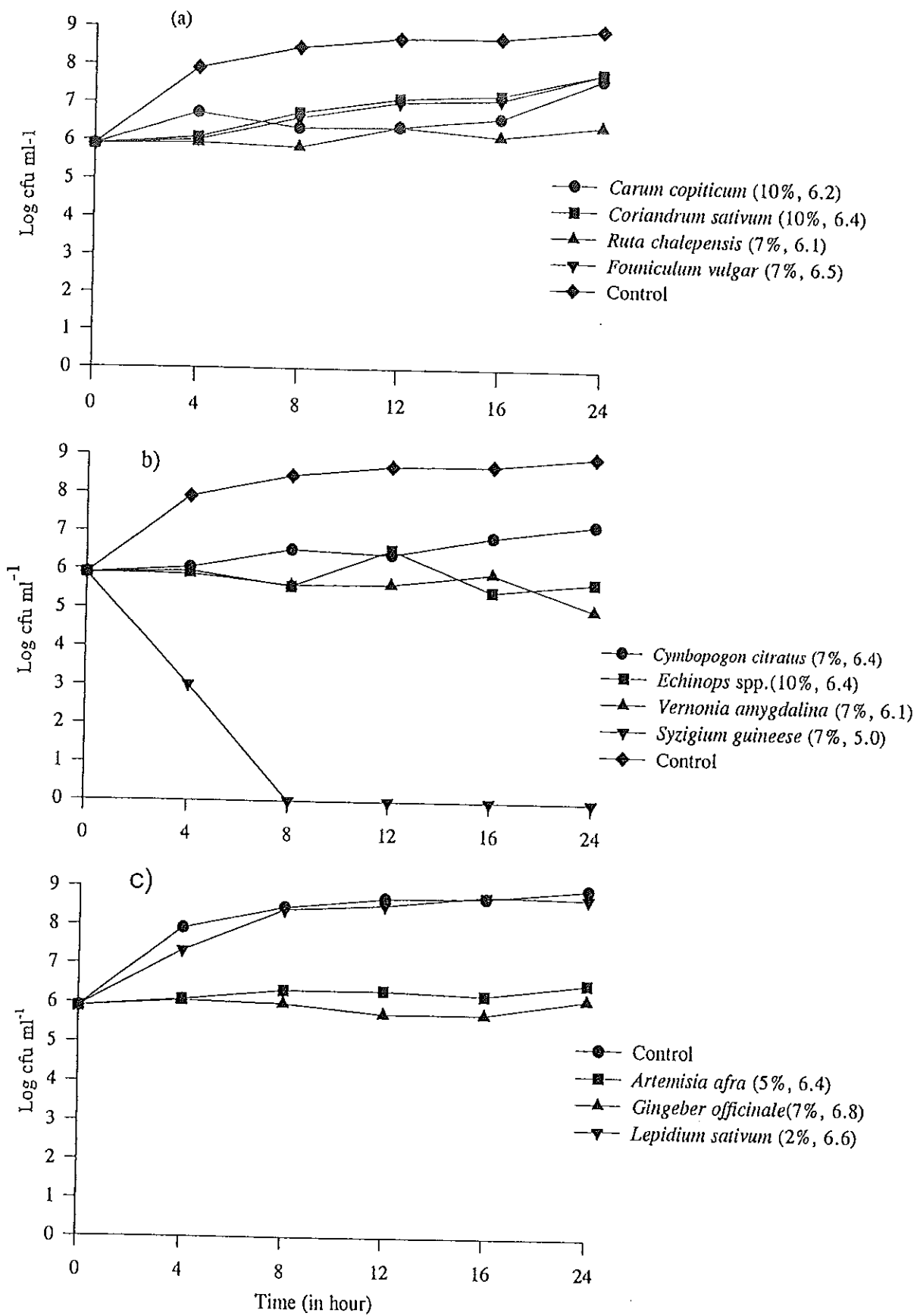


Figure 4 a-c. Response of *Staphylococcus aureus* to various concentrations of the crude preparations of the traditional medicinal plants. values in brackets are weight /volume and pH respectively.

Almost all the crude preparations delayed the growth until 4 h (less by 1 to 4 log units than the control), but final population was not different from the control (Fig. 5).

Of the total crude preparations only two had effect on *Shigella boydii* at the tested concentration. One of these reduced the final population by one log unit. The other was bactericidal to the organism killing the whole population within 8h (Fig. 6)

In all the crude preparations *Escherichia coli* grew very luxuriously. In some five of the crude preparations it grew above the control by one log unit after 24h. Only one, *Syzygium guineense*, delayed the population by one log unit as compared to the control. But even this did not at all affect the final population (Fig. 7)

Salmonella typhimurium, like *Escherichia coli*, grew very luxuriously on all the crude preparations except *Syzygium guineense*. None of them, except in *Syzygium guineense*, which had some effect on the final population. At the early stages, up to 4 and 8 h, most delayed the growth of the organism (Fig. 8).

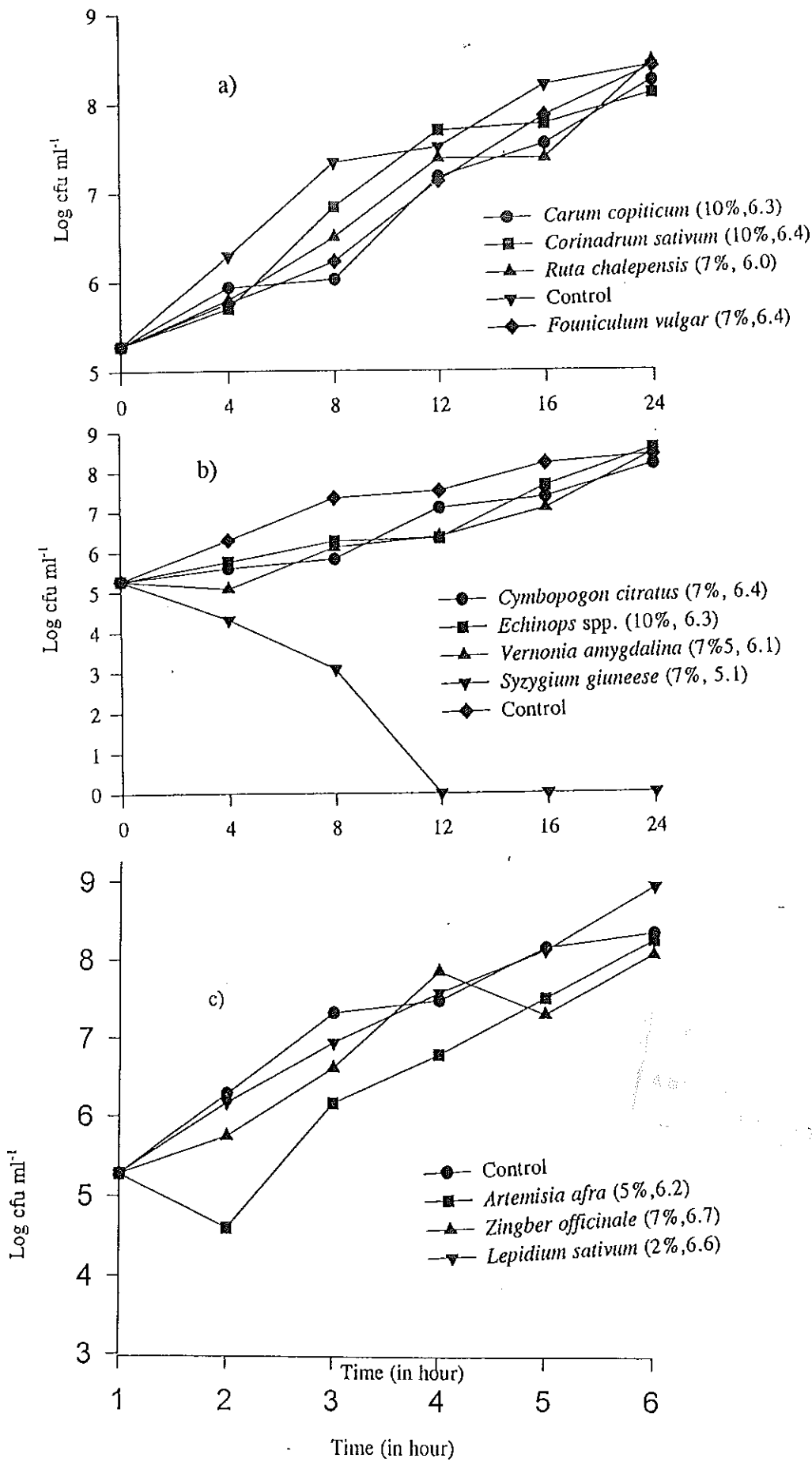


Figure 5 a-c. Response of *Shigella flexneri* to various concentrations of the crude preparations of the medicinal plants. Values in bracket are weight/volume and pH, respectively.

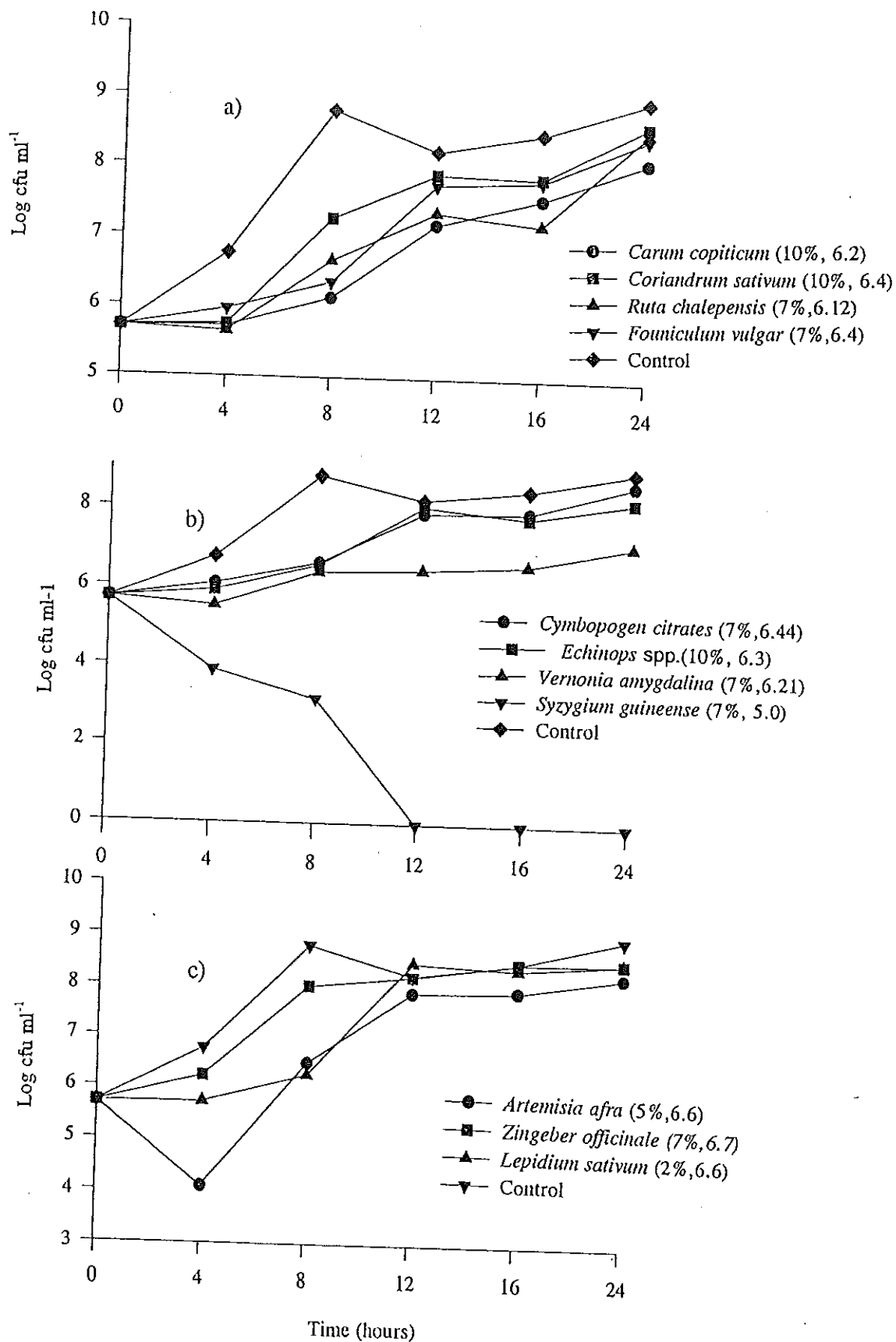


Figure. 6 a-c. Response of *Shigella boydii* to various concentrations of the crude preparations of the medicinal plants. Values in bracket are weight /volume and pH, respectively.

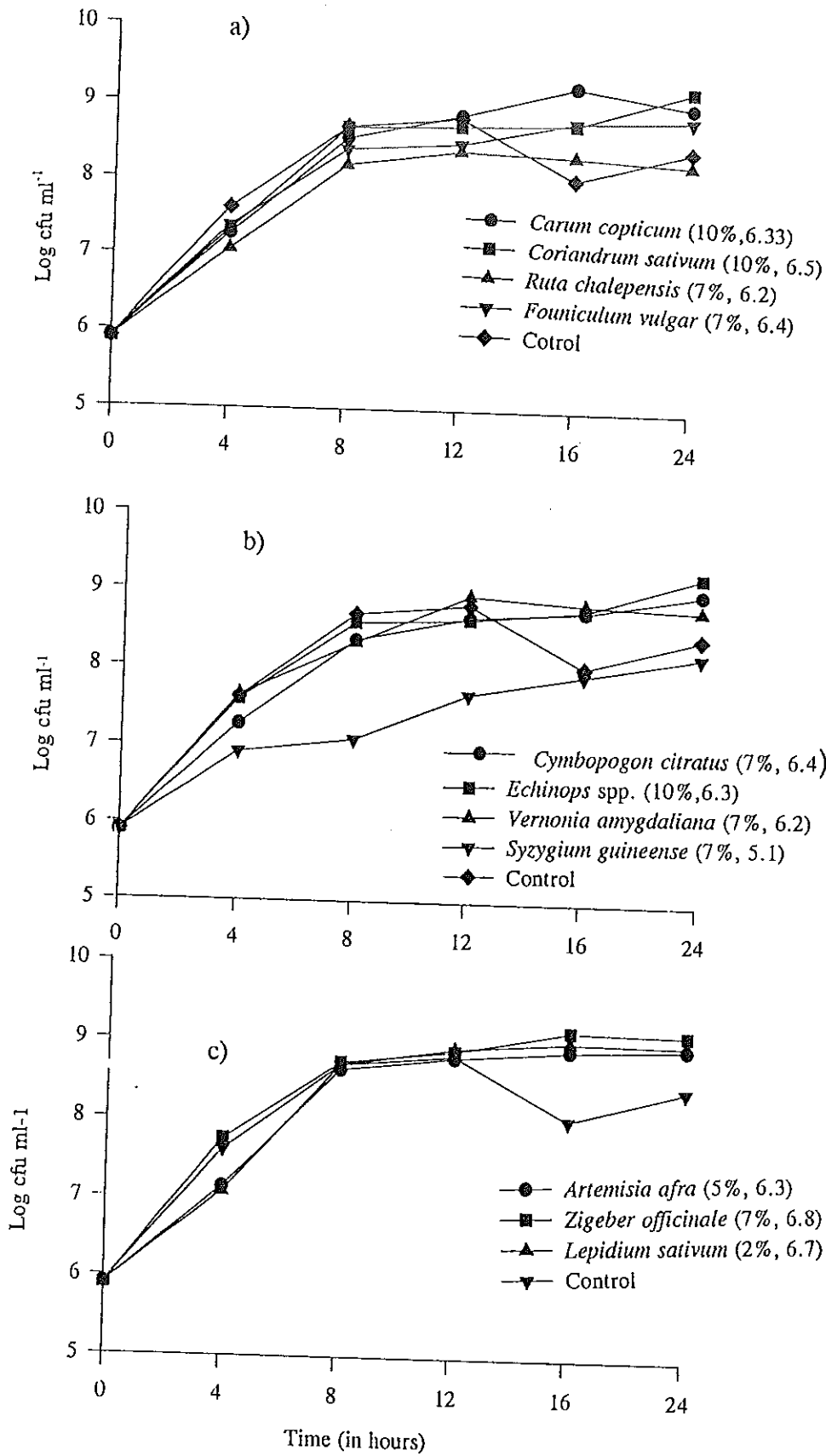


Figura 7a-c. Response of *Escherichia coli* to various concentrations of the crude preparations of the medicinal plants. Values in the bracket are weight/volume and pH, respectively.

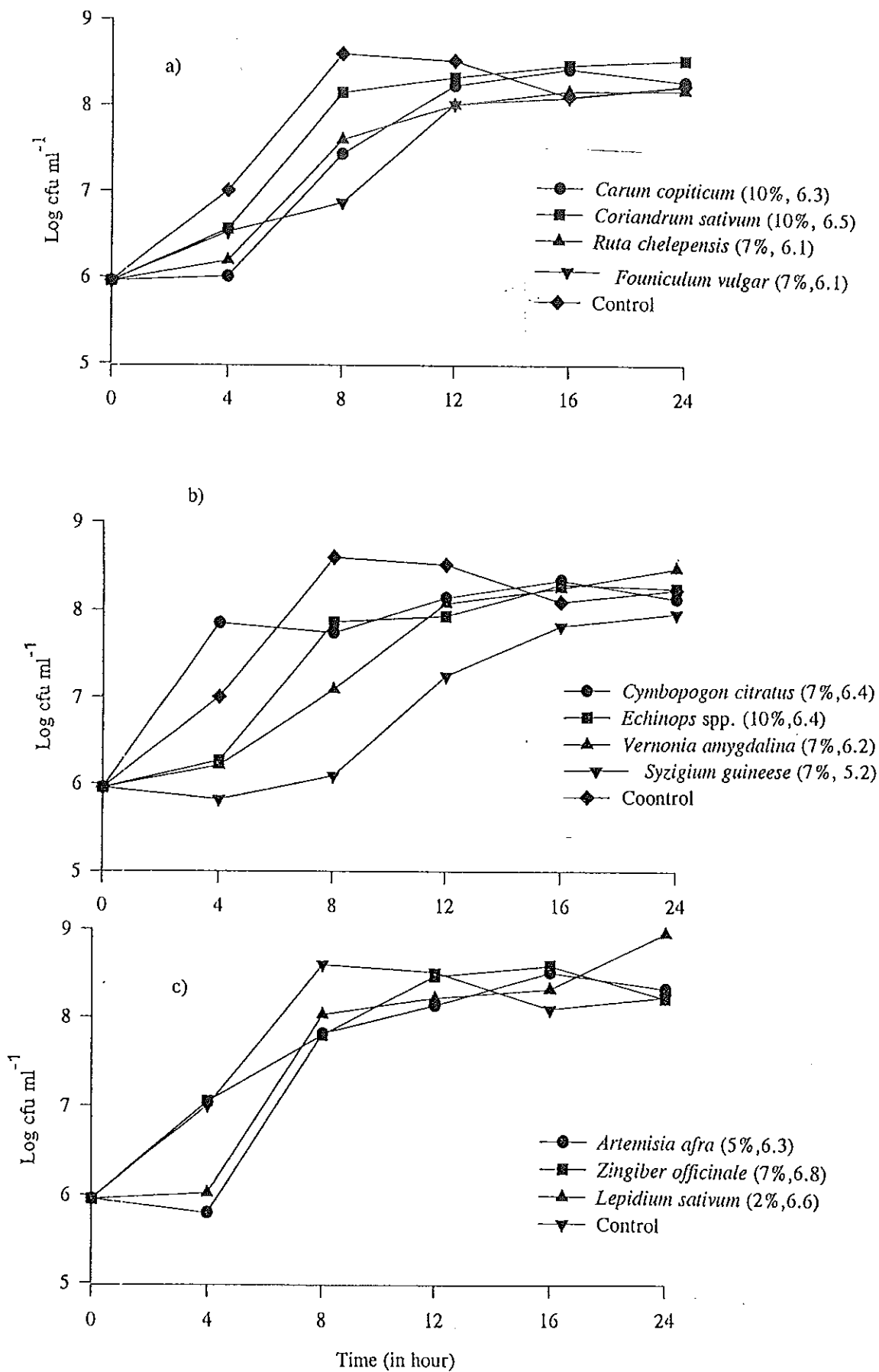


Figure 8a-c. Response of *Salmonella typhimurium* to various concentrations of the crude preparations of the medicinal plants. Values in bracket are weight /volume and pH, respectively.

5. DISCUSSION

In the preliminary investigation with the agar well plate assay, absence of any clear zone by the sterilized and tyndalized crude preparations could be due to either absence or low concentration of diffusible water soluble active constituents or excessive heating that affects thermo-labile biologically active substances. However, the inactivity of most of the preparations at reduced heating up to 40⁰c indicated that heating might not be a factor. The concentration of the active component may, rather, be very low or absent in the crude preparation. The finding of activity with *Echinops* spp. and *Lepidium sativum* by Belachew (1993) supports the latter view. In his investigation, both the methanol and residual aqueous extracts of *Echinops* spp. obtained at a temperature of about 40⁰c, were found effective against *Staphylococcus aureus*; *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella gallinarum*. The methanol extract was found effective on *Salmonella gallinarum* and *Klebsiella pneumoniae*. The residual aqueous and direct aqueous extracts of *Lepidium sativum* were found effective against *Staphylococcus aureus* and the latter was also shown to be effective on *Proteus vulgaris*. The results support the view that the crude preparations of these two traditional plants are ineffective against *Staphylococcus aureus* and *Escherichia coli* due to the low concentration of the active constituent. A similar result has been obtained by Gungnani and Ezenwanzee (1985) using Ginger. The methanol extract of *Zingiber officinale* has shown activity against *E.coli*, *Proteus vulgaris*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Streptococcus faecalis*. Although the variation in solvent might bring a difference, certainly there exists a polar compound active against the indicated organisms. On the other hand, Ndounga *et al* (1994) also found the aqueous lyophilized extracts of *Syzygium guineese* to be effective on *Staphylococcus epidermidis* *Staphylococcus aureus*, *Shigella*

dysenteriae, *Pseudomonas aeruginosa* and *Escherichia coli*. This finding again supports the above mentioned view. However, the lack of activity by the crude preparations may not only be due to low concentration of the active constituent. The active constituent may be absent from the tested plant. A number of factors such as the time of collection, climate etc. might affect the amount of the active constituents in the plant specimen. The active constituents even may be there with enough concentration but might have exerted an antagonistic effect over one another, although current view is in support of the synergistic effect of the crude preparations than the antagonistic effect (personal communication). Earlier it was believed that the heterogeneous phytoconstituents of crude preparations possess either synergistic or antagonistic effect (Farnsworth *et al*, 1966).

The MIC (2.5% W/V) shown by *Ruta chalaepensis* against *Bacillus cereus* with the well plate diffusion assay did not change with change in T^o, but the MIC of *Echinops spp.* (0.625%) changed to 2.5%. The value increased as T^o increased from 40^oc through 62.8^oc to 80^oc and remained unchanged as the T^o progresses to 95^oc. From this data it is clear that the active constituent of *Ruta chalaepensis* may be heat resistant up to 95^oc for 10 minutes. But the active constituent of *Echinops spp.* showed a decrease in activity as the T^o was elevated from 45^oc through 62.8^oc to 80^oc and 90^oc. The latter has been confirmed, although not significant (P =0 .118; r = +0 .8819). But for the former both P and r were 0.

The pH treatment test indicated that the active constituent showed a higher activity (larger diameter of clear zone) at neutral pH. Deviation from neutrality in either side decreased the activity. But when the pH value increased or decreased far away from neutrality to the either extreme, the activity began to raise. This may be due to the fact that increase in OH-

or H⁺ increased the lethal or toxic action of physical and chemical agents (Frobisher, 1968).

The activity of the crude preparations by agar dilution assay might indicate the existence of a water-soluble, non-diffusible active constituent in the crude preparations. The maintenance of their activity both at high T^o (121^oc for 15 minutes) and at pH 7 indicated the stability of the active constituent at high T^o and the presence of an active constituent effective on the susceptible organism without the effect of pH.

Although that majority of the crude preparations showed inhibitory effect, they were effective against Gram positive bacteria with variation in their effective concentrations. About half of the tested crude preparations, at various levels showed activity (inhibition) in one or both the Gram +ve organisms while one has shown activity on both of Gram +ve and Gram -ve. There was no crude preparations which was active only on Gram-ve bacteria.

Of all the crude preparations, *Syzygium guineense* was unique in its ability to inhibit both Gram +ve and Gram-ve bacteria. In fact a similar result has been obtained with lyophilized aqueous extracts of *Syzygium guineense* by Ndounga *et al*, (1994). These authors have suggested to use *Syzygium* aqueous extracts as antiseptic.

In this study, unlike the work of Ndounga *et al* (1994), the crude preparation did not show shown any inhibition on *E. coli* and *Salmonella typhimurium*. This may be because the authors used extract than crude preparation. There is a clue from the fact that *Syzygium guineense* at 7% (w/v) reduced the final population of *Salmonella typhimurium* by one

log unit. Among the plants, it is *Syzygium guineense* that have resulted in low count of *E.coli* after 24h, indicating the slight effect of the crude preparation.

The MIC obtained by agar dilution assay of the various crude preparations varied among plants on the same organism. The variation of the MIC of the various crude preparations on the same organism may be due to either the potency or the amount of active constituent.

The existing most clinically useful antibiotics are active at low concentration. Crude extracts of plants that are active at 100 µg/ml are supposed to be promising (Mitscher *et al.* 1972). When we compare the MIC of crude preparation of *Syzygium guineense* to this figure, it was only 10 times higher. Thus, if the extract is used, one may get a better promising result than that obtained now. Still at the crude level, it is the most promising, not only because of its activity at low concentration, but because of its activity on a wide range of organisms from the two categories (Gram +ve and Gram-ve bacteria).

Unlike *Shigella flexneri* and *Shigella boydii* which responded to higher MBC of *Syzygium guineense*, *Staphylococcus aureus* responded to much lower MBC (1.25%). But in both cases, although there is a wide variation in their MBC, the whole population were eliminated within 8 h.

Some of the plants used as a traditional medicine such as *Carum copiticum*, *Zingiber officinale* and *Coriandrum sativum* are also used as spices in Ethiopia. These plants, especially the first two are inhibitory to *B. cereus*. Thus, at appropriate concentrations, they may reduce the population of *B. cereus* in sauces and prevent it from entering the

logarithmic phase which is thought to be the phase at which *B. cereus* toxin is produced (Hayes, 1995).

In the assessment test, *E. coli* and *Salmonella* grew above the control in some of the crude preparations. Here, the organism might have obtained an additional co-substrate from the crude preparations in addition to the substrate in the growing medium.

6. Conclusion and Recommendations

The result obtained in this preliminary study suggests the fact that more than 70% of the tested crude preparations have effect on both or either of the Gram +ve bacteria tested. There is a drastic effect due to *Syzygium guineense* on both the Gram +ve and specially on *Staphylococcus aureus* and the Gram -ve's particularly on *Shigella flexneri* and *Shigella boydii*. Thus this clearly indicates that some of the traditionally used medicinal plants have an antibacterial effect

Although this work gives evidence about the effect of the crude preparations of the traditional medicinal plants, further study is needed. The samples of the plant in this study have been either bought or collected from the same place at the same time. But it would be advisable if study is conducted on plant materials collected from different areas, climates, soil types, season etc. as all these can affect the abundance of active ingredients in the plant material.

The existence of an active antibacterial effect in an *in vitro* study of the crude preparations, however, does not warrant the safety of the crude preparation. Although that people are using it, there are cases in the community where patients have been affected by consuming crude preparations. A good example in Ethiopia is the ingestion of the crude preparation of *Hagenia abyssinica* 'Kosso' as an antihelminthic drug. Thus this work needs further *in vivo* study to determine the pharmacological effect of the crude preparations. Further the active constituent(s) should be studied very deeply as some plant constituent that are active *in vitro* might negatively affect the host organism.

Inspite of the existence of *Salmonella* and *Eschericia coli* as an agent of diarrhea in the developing countries including Ethiopia and the utilisation of crude preparations of traditional medicinal plants against diarrheal infections by more than 80% of the population in these countries the inactivnes of the majority of the tested crude preparations against the above mentioned food borne pathogens is quite sa discomfort. But the effectiveness of *syzigium giuneense* and the other crud preparations, which are bacteriostatic to the tested organisms is promising.

Although in the *in vitro* test many of the crude preparations are either bacteriostatic or with limited effect on the final population of the test organism or with no effect at all, they may be effective if mechanism have been developed to increase the concentration of the crude preparation more than the concentration tried in this work. Indeed it would be more agreeable with the dose used by the users if it is assesed at large concentrations. one can also say more reliable conclusion based on such data about the use of the medicinal plants.

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