

ORIGINAL ARTICLE**Evaluation of the antibacterial activity of crude preparations of *Zingiber officinale* ("zinjibl"), *Echinops* spp. ("Kebericho"), *Coriandrum sativum* ("dimbilal") and *Cymbopogon citratus* ("tej sar") on some food-borne pathogens.****Mintesnot Ashebir, MSc¹, Mogessie Ashenafi, PhD²**

Abstract: Crude preparations of four types of traditional medicinal plants used in Ethiopia, collected from local markets, were assessed for their antimicrobial activity against some food-borne pathogens. The growth or inhibition pattern of Bacillus cereus, Staphylococcus aureus, Shigella boydii, Shigella flexineri, Salmonella typhimurium and Escherichia coli was determined in growth media separately containing Zingiber officinale (7%), Echinops spp. (10%), Coriandrum sativum (10%) and Cymbopogon citratus (7%). B. cereus was less sensitive to Coriandrum sativum but in media containing Echinops spp., Cymbopogon citratus and Zingiber officinale, final counts were less than initial counts. Although Staphylococcus aureus was not completely inhibited by the crude preparations, counts were less than those of the control at all stages of sampling. The Gram negative test strains were generally less sensitive to activities of crude preparations. Some retarding effect was noted on Shigella flexineri and Shigella boydii until 4 hours. Echinops. spp. was relatively more inhibitory to both Shigella test strains. Growth pattern and counts of Salmonella typhimurium and E. coli at all stages were not different from those of the control.

Introduction

Most food - borne gastro-enteritis are usually self limiting in adults although they could be fatal to infants, the elderly and immune-compromised people. Treatment is, thus, important in serious

cases. But most of the aetiologic agents in many countries have already developed resistance to common antibiotics. Antibiotic resistance by food - borne enteropathogens is also reported in Ethiopia (1,2).

¹ Department of biology, Faculty of science
Addis Ababa University, Addis Ababa

² Institute of Pathobiology
Addis Ababa University Addis Ababa

In most developing countries people live under poor hygienic conditions and, thus, incidence of food-borne diseases is high. The health care system, particularly in the rural areas, is not developed and traditional healers are important in this aspect. About 80% of the world population depends on traditional medicine (3) and use of herbal medicine is popular among the African population. In Zimbabwe, for example, 80% of the population depends on traditional medicine as modern drugs are beyond their reach (4). In Ethiopia, too, a similar proportion of the population depends on traditional medicine (5).

Traditional healers mostly use medicinal plants. Some of these plants, although they are not investigated scientifically, can cure certain infections (6). It can also be assumed that the major part of traditional therapy involves the use of plant extracts or their active principles (3). Investigations made on African (7-9), Asian (10-12) and Latin American (13) medicinal plants have shown the therapeutic importance of the plants whereas some studies could not detect anti-bacterial activity in other herbal medicines (14).

Ethiopia is one of the six countries of the world where about 60% of the plants are said to be indigenous with healing potential (15). However, only few studies have been made regarding indigenous medicine (15). Most such studies concentrated on extracts of the plants and not on the crude preparations, although most of the time traditional treatment uses crude preparations. The purpose of this study was, therefore, to evaluate the antimicrobial potential of the crude preparations of some of the medicinal plants *Zingiber officinale* ("zinjibi"), *Echinops* spp. ("kebericho"), *Coriandrum sativum* ("dimbilal"), and

Cymbopogon citratus ("tej sar") against *Staphylococcus aureus*, *Bacillus cereus*, *Shigella flexneri*, *Shigella boydii*, *Salmonella typhimurium* and *Escherichia coli*. These plants are recorded in the literature as anti-diarrheal (16-17).

Materials and Methods

Sample Collection and Processing: Four types of Ethiopian traditional medicinal plants were either collected from home gardens or purchased from local markets. The seeds of *Coriandrum sativum*, leaves of *Cymbopogon citratus* and root parts of *Echinops* spp. and *Zingiber officinale* were purchased from local markets or collected from gardens.

Samples were thoroughly cleaned with sterile distilled water. The cleaned plant parts were then sun dried, powdered and sieved with a mesh.

Known mass of the prepared powder of each medicinal plant was thoroughly mixed with distilled water to give the maximum concentration that could allow pipetting for microbiological processing. This was sterilized at 121°C for 15 minutes. The maximum concentration of the various plant materials prepared was as follows: *Zingiber officinale* 10%, *Cymbopogon citratus* 10%, *Echinops* spp. 10%, and *Coriandrum sativum* 20% (weight/volume).

Screening for Antimicrobial Activity: For antimicrobial testing the following bacterial strains were kindly supplied by Dr. Abera Geyid of the Ethiopian Health and Nutrition Research Institute (EHNRI): *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Salmonella Typhi-murium* (ATCC 14028), *Bacillus cereus*, *Shigella Flexneri*, and *Shigella boydii*

were from the culture collection of EHNRI.

A loopful of the test strains was separately inoculated into sterile Muller Hinton broth. After incubation for 24 hours at 37°C the cultures were compared with McFarland turbidity standard as described by Thrupp (18) to adjust to a population of 10⁶ cfu/ml.

For screening purposes, powder from the various medicinal plants was separately mixed with Muller Hinton agar at various concentrations, sterilized at 121°C for 15 minutes and poured on sterile petri dishes. Preparations were also similarly processed as follows: boiling for 10 minutes, heating at 80°C for 10 minutes, pasteurization at 62°C for 30 minutes, and lyophilized. A loopful of the standardized culture of each organism was separately streaked on the solidified plates and incubated at 37°C for 24 hours. Inhibition was visually assessed.

Determination of bactericidal activity:

For those crude preparations which showed certain degrees of antimicrobial activity on the test strains, bactericidal activity was determined according to Thrupp (18) with slight modification. Based on information obtained from screening tests, the following concentration of the crude preparations was prepared for determination of bactericidal activity by mixing with 9 ml Mueller Hinton broth: *Cymbopogon citratus* (7%), *Echinops spp.* (10%), *Zingiber officinale* (7%) and *Coriandrum sativum* (10%). After sterilization at 121°C, the broth was separately inoculated with 1 ml of the standardized culture of the test strains to get a final inoculum level of about 10⁵ cfu/ml. A Mueller Hinton broth tube

without plant material served as a control. Appropriate dilutions of freshly inoculated broth was surface plated on Plate Count agar to determine the initial inoculum level. Samples were then drawn at 4 hour intervals and appropriate dilutions were similarly plated for counting after incubation at 37°C for 24 hours.

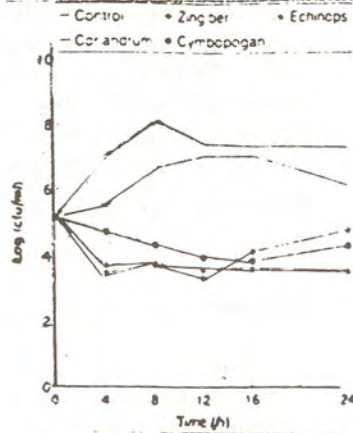
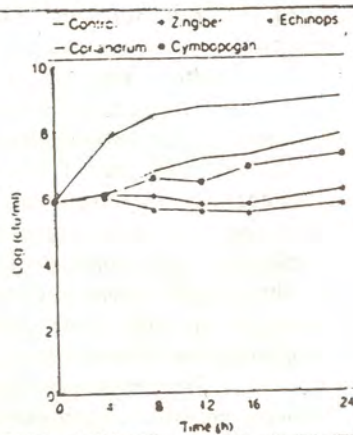
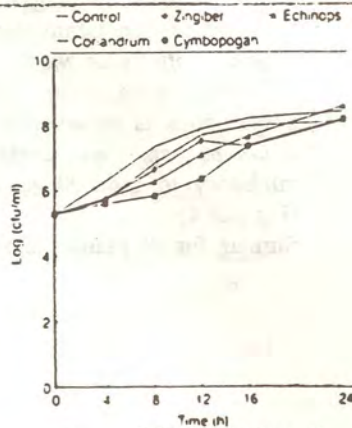
Results

Coriandrum sativum did not show a marked inhibitory effect on *B. cereus* although final count was slightly lower than that of the control. In the case of *Echinops spp.*, *Cymbopogon citratus* and *Zingiber officinale* final counts were less than initial counts and inhibition was steady until 12 hours (Fig 1).

The count of *S. aureus* in broth containing the various crude preparation was less than that of the control at all stages of sampling. In the case of *Coriandrum sativum* and *Cymbopogon citratus* final counts were higher than initial counts whereas decrease in final counts was noted in the case of *Zingiber officinale* on *Echinops spp.* (Fig 2).

The Gram negative test strains were generally less sensitive to activities of the crude preparations. Some retarding effect was noted on *S. flexineri* and *S. boydii* until 4 hours and the count increased thereafter (Fig 3 & 4). Unlike the case on the Gram positive strains, *Zingiber officinale* was relatively less effective than the other crude preparations as observed until 12 hours. *Echinops sp.* was relatively more inhibitory to both *Shigella* test strains (Fig 3 & 4).

Similar for all plants thereafter (Fig 5).

Fig 1. Effect of crude preparations of four medicinal plants on growth pattern of *B. cereus*.Fig 2. Effect of crude preparations of four medicinal plants on growth pattern of *S. aureus*.Fig 3. Effect of crude preparations of four medicinal plants on growth pattern of *S. flexneri*.

All crude preparations did not exhibit any antibacterial activity against *E. coli*. Gro-wth patterns and counts at all stages were not markedly different from those of the control (Fig 6).

Discussion

In screening tests with the agar plate diffusion assay, no clear zones were observed to any of the sterilized crude preparations possibly due to absence of water-soluble active constituents or low concentration of them. The heat treatment (sterilization) of the crude preparations may not have any effect on possible thermo-labile biologically active substances, if any. Most preparations treated with reduced heat (40°C) were ineffective, either. In addition, the fact that most crude preparations in our study showed stronger inhibitory effect against *B. cereus* was indicative that heat treatment did not have any effect on the activity of the crude preparations.

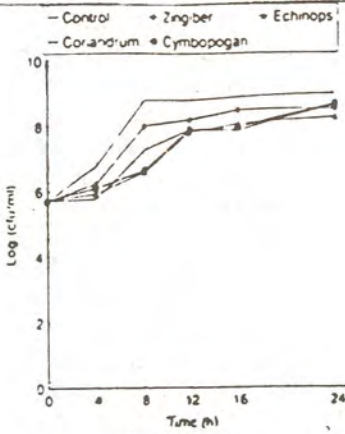
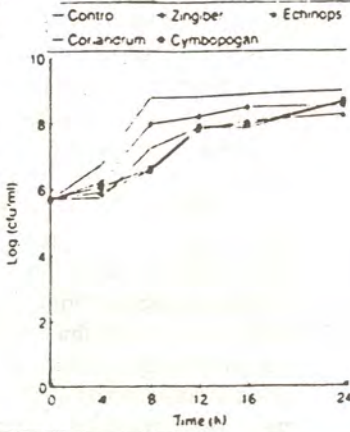
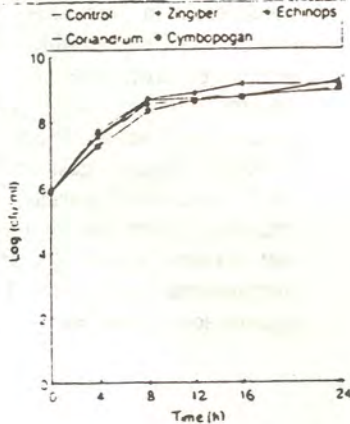
In a similar study, it was reported that methanol extracts of *Echinops* spp. were effective against various Gram positive and Gram negative test strains at a concentration much lower than that for a standard antibiotic (19). In our study, however, *Echinops* spp., although relatively more inhibitory than the other crude preparations, did not show any effective inhibition on most Gram negative test strains. In other studies elsewhere, most of the enteric pathogens were reported to be sensitive to various types of traditional herbal medicines (8,9,12,13), although others, normally considered as having antibacterial effect, were not active in evaluation tests (11,14). Absence of activity in some crude preparations might

also be due to a number of factors such as time of collection of plant material and climate, which might, in turn, affect the amount of active constituents in the plant material.

In many cases extracts of active constituents, which are effective in in-vitro experiments, do not show the same effectiveness when applied in-vivo. This may be due to the fact that various components in the crude preparations may show a synergistic effect on pathogens. According to Farnsworth, et al (20), heterogeneous phytoconstituents of crude preparations may possess synergistic effect.

Except the case with *E. coli* and salmonella the various crude preparations showed varying degrees of retarding effect on all test strains. Given the limitations of our study that crude preparation concentrations were prepared at levels that allowed a broth mixture that can be pipetted for bacteriological analysis, higher concentrations of crude preparations could possibly exhibit marked inhibition as noted in the first four to eight hours in most cases. It is, thus, quite likely that the inhibitory effect of the crude preparations could be considerably enhanced in traditional treatment, if they are taken at four-hour intervals.

The Gram positive species considered in this study are of food intoxication types where toxins rather than vegetative cells are important in causing gastroenteritis. Thus the crude preparations in this study can not be used for treating gastroenteritis caused by *B. cereus* or *S. aureus*. However, the retarding effect shown on our *Shigella* test strains would qualify the crude preparations as potential treatment against some food-borne diseases.

Fig 4. Effect of crude preparations of four medicinal plants on growth pattern of *S. boydii*Fig 5. Effect of crude preparations of four medicinal plants on growth pattern of *S. typhimurium*.Fig 6. Effect of crude preparations of four medicinal plants on growth pattern of *E. coli*.

Acknowledgement

The financial assistance for the first author by SAREC is acknowledged.

References

1. Ashenafi, M. and Gedebo, M. 1985. Salmonella and Shigella in adult diarrhea - prevalence and antibiograms. Trans. R. Soc. Trop. Med. Hyg. 79: 719-721.
2. Assefa, A. and Yohannes, G. 1997. Antibiotic sensitivity of *Staphylococcus aureus* and *Escherichia coli* strains isolated in Gonder, Ethiopia. Trop. Doc. 27: 121.
3. Akerele, O. 1993. Nature's medical botany do not throw it away. World Health Forum. 14: 390-395.
4. Nyazema, Z. N. and Kire, F.C. 1986. The interaction between traditional medicine remedies used for gastrointestinal diseases as conventional drugs. East Afr. Med. J. 63(4): 258-262.
5. Abebe, D. 1996. The development of drug research. EHNRI News Letter. 1: 5-6.
6. Andrews, J. 1982. A bibliography on herbs, herbal medicine, "natural" foods, and unconventional medical treatment. Libraries unlimited, Inc., USA.
7. Chinemana, F., et al. 1985. Indigenous plant remedies in Zimbabwe. J. Ethnopharmacol. 14: 159-172.
8. Maikere-Faniyo, R., et al. 1989. Study of Rwandese medicinal plants used in the treatment of diarrhoea J. Ethnopharmacol. 26: 101-109.
9. Fabry, W., et al. 1998. Antibacterial activity of East African medicinal plants. J. Ethnopharmacol. 60: 79-84.
10. Chowdhury, A.K, et al. 1991. Efficacy of aqueous extract of garlic and allicin in experimental shigellosis in rabbits. Indian J. Med. Res. 93: 33-36.
11. Haider, R, et al. 1991. Evaluation of indigenous plants in the treatment of acute shigellosis. Trop. Geogr. Med. 43: 266-270.
12. Vijaya, K, and Ananthan, S. 1997. Microbiological screening of Indian medicinal plants with special reference to enteropathogens. J. Altern. Complement. Med. 3: 13-20.
13. Careres A, et al. 1993. Plants used in Guatemala for the treatment of gastrointestinal disorders. 3. Confirmation of activity against enterobacteria of 16 plants. J. Ethnopharmacol. 38: 31-38.
14. Leelarasamee, A, et al. 1990. Trakulsomboon S, Sittisomwong N. 1990. Undetectable antibacterial activity of *Andrographis paniculata* (Burma) wall. J. Med Assoc Thai. 73: 299-304.
15. Kaba, M. 1996. Utilization of plant medicine for the treatment of health problems: The case of Oromo, Illubabor Zone, Western Ethiopia. Ethiop. J. Health Dev. 10: 161-162.
16. Edwards, S. and Asfaw, Z. 1992. Plants used in African traditional medicine as practised in Ethiopia and Uganda. Botany 2000: East and Central Africa Monograph Series Number 5. Published by NAPRECA, Addis Ababa University, Addis Ababa.

17. Kloos, H., et al. 1978. Preliminary study of traditional medicinal plants in nineteen markets in Ethiopia: use patterns and public health aspects. *Ethiop. Med. J.* 16: 33-43.
18. Thrupp, D. 1980. Susceptibility testing of antibiotics in liquid media. In: *Antibiotics in laboratory medicine*. Lorian, V. (ed). Williams and Wilkins, USA. PP 73-93.
19. Desta, B. 1993. Ethiopian traditional herbal drugs. Part II. Antimicrobial activity of 63 medicinal plants. *J. Ethnopharmacol.* 39: 129-139.
20. Farnsworth NR, et al. 1966. Biological and phytochemical evaluation of plants. I. Biological test procedures and results from two hundred accessions. *Lloydia.* 25: 101-122.