Original article

Assessment of the antibacterial activity of Some traditional medicinal plants on some food-borne pathogens

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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 of *Bacillus cereus, Staphylococcus aureus, Shigella boydii, Shigella flexineri, Salmonella typhimurium, and Escherichia coli* was determined in growth media separately containing *Artemisia afra* (5%), *Vernonia amygdalina* (7%), *Lepidium sativum* ((2%) and *Carum copticum* (10%). None of the test organisms was affected by *Lepidium sativum* in 24 hours. *B. cereus* and *Staph. aureus* had markedly lower final counts in media containing crude preparations of *Vernonia amygdalina, Carum copticum*, and *Artemisia afra* when compared to control. Retarding effect was noted on *Sh. Flexineri* and *Sh. Boydii* in the initial stages by *Vernonia amygdalina* and *Artemisia afra*. Counts of *S. typhimurium* in all crude preparations were lower by about one log unit than the control until eight hours. None of the crude preparations had any effect on *E. coli*. The antimicrobial effect of some of the crude preparations may be considerably enhanced in traditional treatment if they are taken at four hour intervals. [*Ethiop. J. Health Dev.* 1999;13(3):211-216]

Introduction

The majority of the food-borne diseases, although self limiting, can also be life-threatening needing antibiotic therapy. But most of the aetiologic agents in many countries have already developed resistance to common antibiotics. This type of resistance is also reported in Ethiopia (15).

In most developing countries where people are living in poor hygienic conditions, the chance of contracting food borne diseases is undoubtedly high. Due to absence of sufficient modern health care system, particularly in the rural areas, people prefer to visit traditional healers. According to Akerele Zimbabwe, for (6), 80% of the world population depends on traditional medicine. Thus they depend on traditional medicine to treat themselves. For example, 80% of the population depends on
traditional medicine (7). In Ethiopia, too, a similar proportion of the population also rely on herbal medicine (8).

Some traditional healers use medicinal plants. Some of these plants, although they are not investigated scientifically, can cure certain infections (9). It can also be assumed that the major part of traditional therapy involves the use of plant extracts or their active principles (6). With proper investigation these may serve as a source of modern drug. Drugs such as quinine, digitalis etc. were synthesized from medicinal plants (10). They served also as a source of intermediate compounds for synthesizing analog drugs with more desirable properties (9). In developing countries their use has helped to substitute imports of drugs, thus boosting economic self-reliance. Further more local products tend to be more readily accepted than those obtained from abroad (6).

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and one family of fern are known to contain species with medicinal properties (12).

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Among African countries, Ethiopia is often quoted as one of the six countries of the world where about 60% of the plants are said to be indigenous with healing potential (11). There are about 213 families of flowering plants in Ethiopia and of these 92 families, with one family of gymnosperm

But despite all these virtues, only few studies have been conducted regarding indigenous medicine (11). Most of such studies concentrated on extracts of the plants and not on the crude preparations, although traditional treatment usually considers crude preparations. The purpose of this study is therefore to evaluate the antimicrobial potential of the crude preparations of some of the medicinal plants, Viz. *Artemisia afra*, "Ariti"; *Vernonia amygdalina*, "Girawa"; *Lepidium sativum*, "Feto"; and *Carum copticum*, "Nech Azmud" against *Staphylococcus aureus, Bacillus cereus, Shigella flexineri, Shigella boydii, Salmonella typhimurium* and *Escherichia coli*, respectively. These plants are recorded in the literature as anti-diarrheal (12, 13).

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 *Carum copticum*, *Lepidium sativum*, and leaves of *Artemisia afra* and *Vernonia amygdalina* 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. The concentration of the various plant materials thus prepared was as follows:- *Carum Copticum* 20%; *Vernonia amygdalina* 10%, *Artemisia afra*, and *Lepidium sativum*, 5%(weight/volume). The preparations were separately sterilized at 121°C for 15 min.

Screening for antimicrobial activity: For antimicrobial testing the following bacterial strains were kindly supplied by Dr. Aberra Geyid of the Ethiopian Health and Nutrition Research Institute (EHNRI): *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Salmonella Typhimurium (ATCC* 14028). *Bacillus cereus, Shigella Flexineri,* and *Shigella boydii* were from the culture collection of EHNRI.

A loopful of the test strains was separately inoculated into sterile Mueller Hinton broth. After incubation for 24h at 37°C, the cultures were compared with McFarland turbidity standard as described by Thrupp (14), to adjust to a population of 10^6 cfu/ml.

For screening purposes, powder from the various medicinal plants was separately mixed with Mueller Hinton agar at various concentrations (20%, 10%, 5% and 2.5%), sterilized at 121°C for 15 minutes and poured on sterile petri dishes. Preparations were also similarly processed as follows: boiling for 10 min., heating at 80°C for 10 min., pasteurization at 62°C for 30 min. and lyophilization. A loopful of the standardized culture of each organism was separately streaked on the solidified plates and incubated at 37°C for 24h. Inoculation on plates without medicinal plant preparations served as control. Inhibition was assessed in terms of reduction of bacterial colonies in comparison to control.

Determination of bactericidal activity: Minimum concentrations of crude preparations which showed inhibition in the screening tests were further considered for the determination of bactericidal activity on the test organisms according to Thrupp (14) with slight modification. Thus the following conce-ntrations of the crude preparations were made by mixing with 9ml Muller Hinton broth: *Artemisia afra* (5%, pH 6.6), *Vernonia amygdalina* (7%, pH 6.2), *Lepidium sativum* (2%, pH 6.7), and *Carum copticum* (10%, pH 6.3). After sterilization at 121°C, the broth were separately inoculated with 1 ml of the standardized culture of the test strains to get a final inoculum level of about 10^5 cfu/ml. A Muller 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 four hour intervals and appropriate dilutions

were similarly plated for counting after incubation at 37°C for 24h. This study was undertaken in Addis Ababa between October and April, 1998.

Results

Lepidium sativum did not exhibit an inhibitory property against *B. cereus* (Fig 1). Growth in *Lepidium sativum* was almost identical with that in the control. *Vernonia amygdalina* was relatively more inhibitory to *Staph. aureus* than the other plants (Fig 2). Bacteriostatic property of *Carum copticum*, *Artemisia afra* and *Vernonia amygdalina* was observed after 4h, although growth in *Carum copticum* increased after 16h.

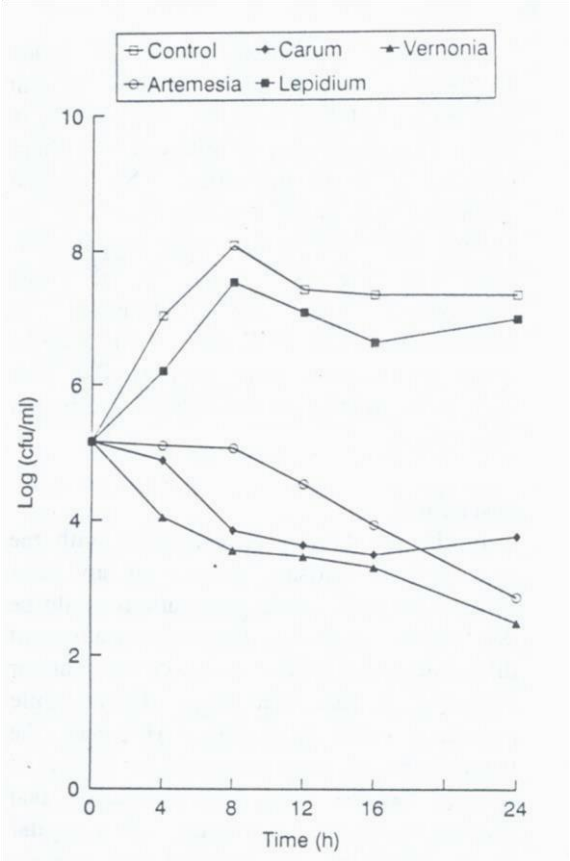

Figure 1: **Effect of crude preparations of four medicinal plants on growth pattern of *B. cereus*.**

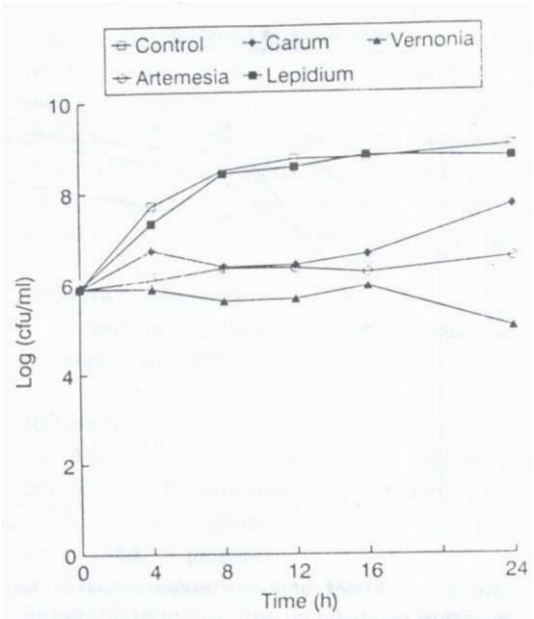

Figure 2: **Effect of crude preparations of four medicinal plants on growth pattern of *Staph. aureus.***

Both *Sh. Flexineri* and *Sh. Boydii* showed similar increasing trends in all crude preparations. *Vernonia Amygdalina* and *Artemisia afra* showed a marked initial retarding effect against *Sh. Flexineri* (Fig 3). Final counts in all cases were, however, higher

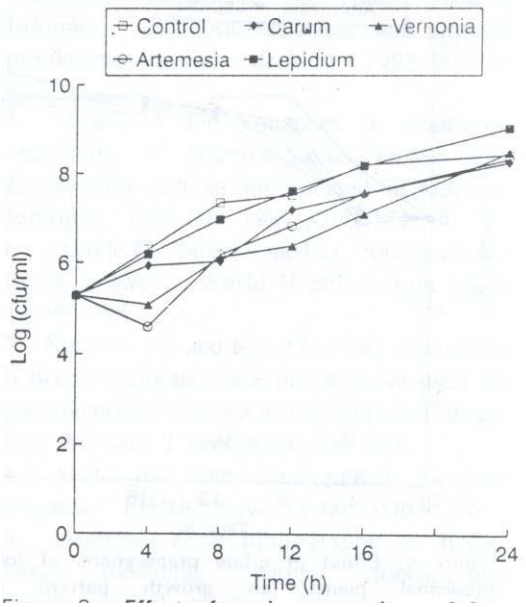

Figure 3: **Effect of crude preparations of four medicinal plants on growth pattern of *Sh. Flexineri.***

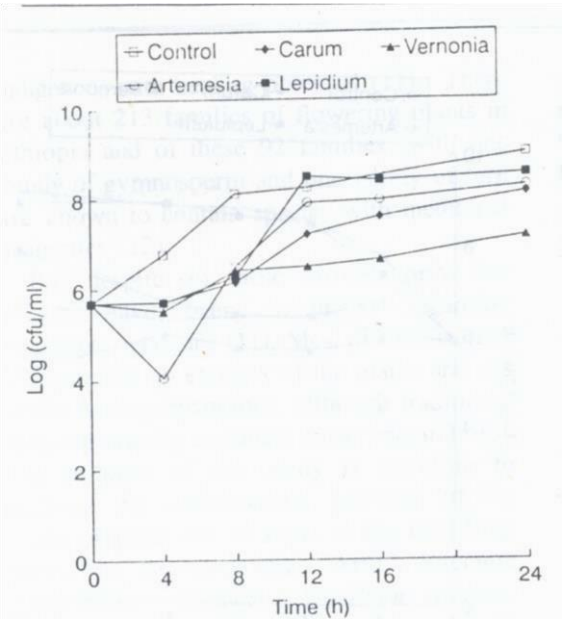

Figure 4: **Effect of crude preparations of four medicinal plants on growth pattern of *Sh. Boydii.***

than 10^9 cfu/ml. *Artemisia Afra* reduced the initial counts of *Sh. Boydii* by about 2 log units within four hours. The other crude preparations also had a bacteriostatic effect until 4h. Final counts in *Vernonia Amygdalina* were all lower by about 2 log units than the control (Fig 4).

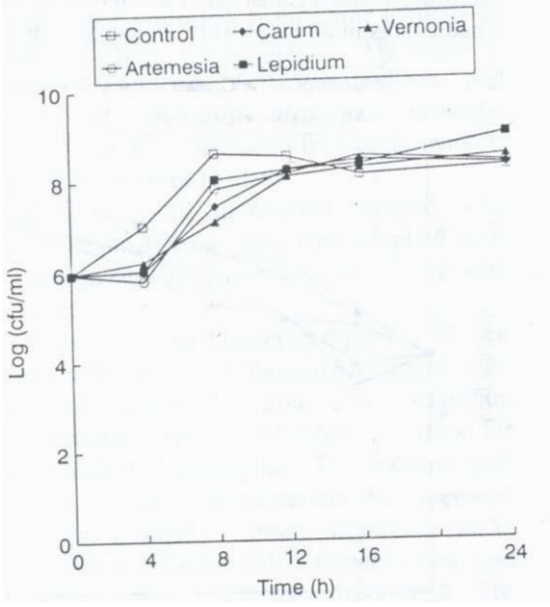

Figure 5: **Effect of crude preparations of four medicinal plants on growth pattern of *S.typhimurium*.**

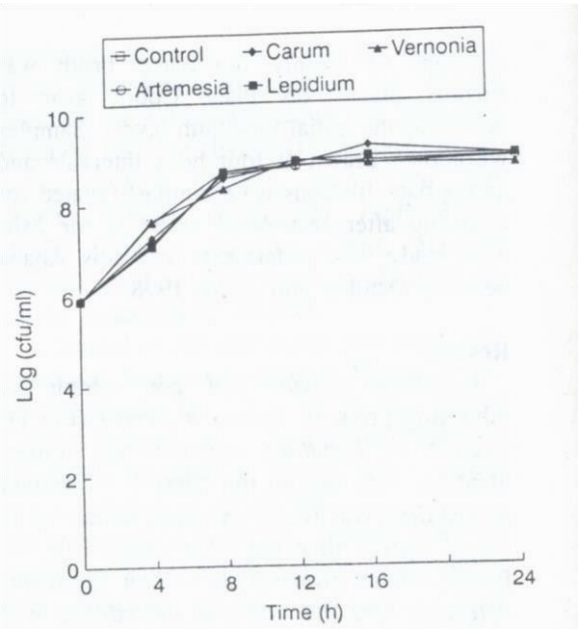

Figure 6: **Effect of crude preparations of four medicinal plants on growth pattern of *E. coli.***

Counts of *S.Typhimurium* in all crude preparations were lower by about 1 log unit than the control until about 8h. All resulted in a longer lag phase until 4h followed by a sharp increase in count thereafter. No marked difference was noted between counts in the control and those in the crude preparations after 12h (Fig 5). None of the crude preparations had any bactericidal or bacteriostatic effect on *E. coli*. Counts in the crude preparations were very similar with those in the control all through the incubation time (Fig 6).

Discussion

In the preliminary investigation with the agar well plate assay, absence of any clear zone by sterilized crude preparations could be due to either absence or low concentrations of diffusible water soluble active constituents or excessive heating that affect thermo-labile biological active substances. However, the inactiveness of most preparations treated at reduced heating upto 40°C indicated that heating might not be a factor. In a similar study, Belachew (15) reported that aqueous extracts of *Lepidium sativum* were found effective against *Staph. aureus* and *Proteus vulgaris*. The absence of inhibition in our study may thus be due to low concentration of active constituents. The fact that some crude preparations in our study showed stronger retardation effect on the Grampositive test strains than on the Gram-negative ones supported our argument that heat treatment did not have effect on the activity of the crude preparations. In another study, Gram-negative test organisms were less susceptible to extracts than Gram-positive strains (15). 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.

Except for *E. coli*, the various crude preparations showed varying degrees of retarding effect on all test strains. A shortcoming of our study was to limit crude preparation concentrations to levels that allowed a broth mixture that can be pipetted for bacteriological analysis. If higher concentrations of crude preparations could be studied, a stronger inhibition might have been noted. 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. Thus, treating diarrhoea caused by food intoxication with the various crude preparations may not be effective at all. Once these pathogens elucidate a sufficient amount of toxin in the food in which they multiply,

it is the toxin, and not the pathogens, that cause the disease after ingestion. However, the retarding effect shown in some of the Gram-negative test strains would qualify the crude preparations as candidates for further research. It may also be important to consider the crude preparations themselves in the study of their medicinal value. 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*., (16), heterogenous phytoconstituents of crude preparations may possess a synergistic effect.

Acknowledgements

Authors kindly acknowledge the financial assistance by SAREC.

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