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Screening of extracts from medicinal plants of Cameroon for antimicrobial activity /

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SCREENING OF EXTRACTS FROM MEDICINAL PLANTS OF CAMEROON FOR ANTIMICROBIAL ACTIVITY

A Thesis Presented

by

VICTOR T. KWO

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ABSTRACT

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MAY 1996

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A disk diffusion susceptibility test was used to screen concentrated extracts from the bark of three Cameroon medicinal plants(*Alstonia boonei, Kigelia africana, Morinda lucida)*for antimicrobial activity. Solvents with different polarity, methylene chloride(non polar),ethyl acetate(slightly polar),ethanol(moderately polar)and acetonitrile(moderately polar)were used for extraction. The extracts were tested against five human pathogens,*Candida albicans* ATCC14043,*Enterococcus faecalis,* ATCC33185, *Staphylococcus aureus* ATCC25923,*Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853.

The patterns of inhibition observed varied with the plant extract,the solvent used for extraction and the organism tested. Ethanol and ethyl acetate extracts were the most active. The largest zone of inhibition was observed with ethanol extracts of *Kigelia africana* against

Staphylococcus aureus and *Pseudomonas aeruginosa.* *Staphylococcus aureus* was the most inhibited microorganism. No inhibition was observed against *Candida albicans.* The extent of the inhibition observed was determined by the concentration of the plant extract.

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CHAPTER I

INTRODUCTION

Plants were used for the treatment of diseases long before any scientific explanations of bioactivity emerged (Chiej 1984). The information gained throughout history is, however, in danger of being lost because it is passed orally from generation to generation (Schultes 1978),and the present generation is losing interest and no written records are available.

Plants are used in the treatment of diseases because they contain chemical constituents which modify body chemistry, making the body more resistant to disease and inhibiting the growth of disease organisms. Indeed plants are a major source of drugs. In the United States, over one quarter of the 1,500 million prescriptions dispensed annually are derived from plants(Ayensu 1978). In Germany, half of the prescription drugs are initially derived from raw plant materials (Ayensu 1978).

Due to a lack of facilities, only a limited number of investigations on medicinal plant use in Africa has been

completed yet in many African countries the soaring cost of synthetic drugs and pesticides underscores a need for the use of plant material for medicine. Scientific evaluation of bioactivity is however necessary to establish efficacy.

Microbiological evaluations of plants and plant extracts has led to the discovery of important active principles. Vinblastine sulphate used as a cure for Hodgkins disease and vincristine sulphate used for lymphocytic leukemia are compounds isolated from the Madagascar periwinkle *Vinca rosea*(Miller 1996,Stehlin 1990). Reserpine, isolated from the roots of tropical plants in the genus *Rauvolfia* is a sedative and used for high blood pressure(Shehlin 1990). Researchers in the National Cancer Institute in an anti-HIV screen reported an active principle from the bark of the Samoan tree *Homalantus acuminatus* (Shehlin 1990).

As deforestation of tropical rainforest continues the need grows most urgent for scientific evaluation and preservation of African medicinal plants before the present population is lost(Macfoy and Cline 1990).

CHAPTER II

LITERATURE REVIEW

Humans have always been interested in the isolation and identification of active chemical constituents from plants (Odebeyi and Sofowora 1978). About 4000-9000 naturally occurring antibiotics have been reported from microbes. This number is even greater if the semi-synthetic antibiotics are considered (Mitscher and Rao 1984).

Several methods can be used to test crude plant extracts for antimicrobial activity (Jansen et al 1986). The appropriate technique and solvent, however has to be used to extract the antimicrobial compounds. Some extraction techniques may destroy the antimicrobial compounds and the polarity of the liquid used for extraction determines the type of compound identified(Stiffler et al unpublished). Vinblastine, the antitumor alkaloid isolated from *Vinca rosea* for example, is insoluble in water but highly soluble in alcohols, acetone, and ethyl acetate. The antibiotic Vancomycin, is however soluble in water and insoluble in higher alcohols, acetone, and ether(Windholz et al 1983).

Rios et al, (1987) screened the chloroform and methanol extracts from eighty-one medicinal plants from the Spanish Mediterranean for antimicrobial activity using the agar

dilution method. Six microorganisms were used and growth inhibition, observed from thirty extracts, was due to the presence of flavonoids, terpenoids, and phenolic acids. Aguwa and Lawal (1988) observed the leaf extracts of *Cellandra portoricensis* to be active against *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus faecalis*. Herbalists in Southern Nigeria use the leaf extracts of this plant for gastrointestinal troubles (Aguwa and Lawal 1988).

Paulo et al (1994) screened the aqueous and ethanolic extracts from the roots of *Cryptolepsis sanguinolenta* using a two fold serial broth microdilution assay. The extracts inhibited growth in all strains of the test organism except *Pseudomonas aeroginosa*. Cryptolepine, cryptoheptine, and indole alkaloids were identified as the active compounds.

Sofowora et al,(1975) isolated 2-hydroxy methyl benzoic acid as the antisickling agent in *Fagara zanthoxyloides*. Odebiyi and Sofowora (1979) reported the antimicrobial activity of this plant was due benzoic acid derivatives and alkaloids. The roots and stems of this plant are used by Africans as chewing sticks against oral microbial flora. (Sofowora 1982). Tafur et al, (1976) identified camptothecin and 10-methoxycamptothecin as the antiviral component of *Ophiorrhiza mungos*. Acharya and Chatterjee (1975), working on the defatted seed powder of *Cassia tora*, identified Chrysophanic acid-9-Anthone, chrysophanic acid,

and hydroxyanthraquinone derivatives as the antifungal agents.

Osborn (1943), working in England, reported a comprehensive screening program using the diffusion method involving 2300 plant species from 166 families. *Staphyloccus aureus* and *Escherichia coli* were the test organisms. Sixty-three genera from twenty-eight families showed activity against one or both of the test organisms.

Irobi and Banso (1994) demonstrated the antibacterial activity of *Acalypha torta* using four anaerobic bacteria. Growth inhibition was observed in all the anaerobes used in the study but the *Clostridia* were more susceptible. Phytochemical analysis of the leaves indicated the presence of phenols, tannins, saponins, and sesquiterpenes. Mensah et al, (1990) demonstrated the bacterial action of the alkaloids viroallosecurine and securine from the lyophilized aqueous leaf extract of *Phyllanthus discoideus* from Ivory coast. Odebiyi and Sofowora (1978) reported the presence of alkaloids, saponins, tannins, phlobotannins, and anthraquinones after screening forty-seven extracts from Nigerian traditional medicinal plants.

Alstonia boonei, Kigelia africana and *Morinda Lucida* are medicinal plants widely used in traditional medicine in Cameroon. *Alstonia boonei* De Wild, synonym *Alstonia congensis,* (family Apocynaceae) is a tree which attains a

height of 40 meters(Burkill 1985) with leaves that are elongate-oblanceolate, round-acuminate, occurring in whorls (Irvine 1961). The inflorescence is terminal (FAO 1986) and the flowers are small and white, flowering from November to February. The fruits are paired (Irvine 1961).

In traditional African medicine, the latex of this plant is used for skin infections and, in combination with the crushed bark of *Erythrophleum guineense,* appears to have anti-inflammatory activity. The latex, mixed with palm wine, is galactogenic (Burkill 1985, Oliver-Bever 1986). A decoction of the bark is antimalarial, antirheumatic, a vermifuge, and cures asthma. Taken after childbirth,the decoction assist the expulsion of the placenta (Irvine 1961, Burkill 1985). In Ivory Coast, the plant is used as a febrifuge and as a cure for gonorrhea. The leaf pulp is anti-inflammatory. A decoction of the bark is a remedy for wounds and open fractures (Kerharo and Bouquet 1950).

In Nigeria, this plant is used for treatment of sores and ulcers and, in combination with other herbs, is used for treatment of asthma(Irvine 1961). The latex is an antidote for *Strophanthus* poisoning(Irvine 1961). A few drops of the sap of *Anthostema aubryanum* added to the latex is used as a purgative (Irvine 1961). *Alstonia boonei* is also used as an analgesic, an antidote for snakebite, and arrow poison (Burkill 1985). An infusion of the stem bark and fruit of

Piper guineense in locally distilled gin is drunk once daily to treat impotence (FAO 1986).

Kweifo-Okai (1991) noted the aqueous infusion of *Alstonia boonei, Rauvolfia vomitoria* and *Elaies guineensis* active in suppressing the late phase of carrageenin edema in rats. This plant combination is used in Ghanaian folk medicine for rheumatoid arthritis. *Alstonia boonei* constitutes 90-95% of the mixture and has many constituents (Table 1).

Table 1. Reported constituents of *Alstonia boonei*

Constituent	Reference
Echitamidine	13,26
N-formylechitamidine	26
12-methoxy-n-formylechitamidine	26
Echitamine	13
Nor-echitamine	13
17-acetoxy-*nor*-echitamine	13
Akuammicine	13
12-methoxyakuammicine	13
12-methoxy-n(4)-methylakuammicine	13
Tubotaiwine	13
12-methoxytubotaiwine	13
Angustilobines A and B	13
6,7-secoangustilobine A and B	13
Angustilobine b-n(4)-oxide	13
Akuammidine	13
Boonein	25

Some phytochemical evaluation has been done on other species in the genus *Alstonia*. Allam et al, (1987) identified a new alkaloid 14-ketoalstonidine and eight other alkaloids from the stem bark of *Alstonia constricta* from Melbourne, Australia. Yamauchi et al (1990), working on

Alstonia scholaris from the Philippines, isolated Echitamine and 17-0-Acetylechitamine from the bark and six other alkaloids from the leaves.

Kigelia africana (Lam) Benth [Synonyms:*Kigelia pinnata* (Jacq.)DC,*Kigelia aethiopica* Decne,*Kigelia abyssinica* Rich,*Kigelia elliottii* Sprague] (family Bignoniaceae) is commonly known as the sausage tree and is locally known in the South west Province of Cameroon as Musong. This tree is 24 meters tall with a low branching trunk and alternate pinnate leaves. Stipules are absent (FAO 1986). The inflorescence is a lax pendulous panicle 90 cm long. The flowers are hermaphrodite and the fruits are sausage shaped, grayish, and indehiscent(FAO 1986).

An infusion of the stem bark with other herbs and guinea grains is used in traditional african medicine as a treatment for venereal diseases. The bark is apparently antirheumatic and provides a remedy for dysentery and wounds (Irvine 1961). The bark is also used as an enema and with *Tateorhiza,* as an antidote for snakebite. (Burkill 1985). A decoction of the stem bark is used as an emmenagogue (FAO 1986) and is combined with the leaves of *Irvingia gabonensis* to cure spleen infections(FAO 1986).

The fruit and root, boiled with the stem and tassel of plantain is used to cure post-parturition hemorrhage. The powdered fruits with palm oil are a remedy for dizziness

(FAO 1986). In Ivory Coast the fruit is used for scrotal elephantiasis and oedema of the legs (Bouquet and Debray 1974).

The leaves of the plant are used for dysentery, stomach trouble, kidney infections, and snakebites (Irvine 1961). A decoction of the leaves and stem bark is antipyretic (FAO 1986)and paste made from the fruit is applied to boils (Burkill 1985). Akunyili et al, (1991) found the aqueous extracts of *Kigelia africana* to have growth inhibitory effects on five microorganisms. *Kigelia africana* has many constituents(Table 2).

Morinda Lucida Benth (synonym *Morinda citrifolia* Chev.) (family Rubiaceae) is commonly known as the Brimstone tree. The plant reaches a height of 50 feet, and has a dense crown. The leaves are broadly elliptic to ovate, acuminate, and entire. The flowers are white and occur as terminal and axillary heads on peduncles (Irvine 1961).

A decoction of the bark of *Morinda lucida* is drunk for piles and dysentery and the pounded bark and root is used as an enema for constipation (Irvine 1961). The root bark is a diuretic (Oliver-Bever 1986). A decoction of the root is used as an emmenagogue, to cure gonorrhea, and in combination with the leaves, as an anti-abortive (Irvine 1961). Stem scrapings placed on the abdomen are oxytocic (Ayensu 1978). The stem bark, leaves, and roots are

reported to have astringent, antimalarial, and antipyretic activity and provides remedy for dysentery, jaundice, and yellow fever (Oliver-Bever 1986).

A leaf decoction is used as a depurative and to relieve constipation. A leaf infusion with other herbs is used as a tonic for infants. *Morinda lucida* is reported to cure leprosy and act as an antidote for poison (Irvine 1961). Asuzu and Chineme (1990) reported trypanocidal activity from methanolic extracts of the leaves of *Morinda lucida*. Phytochemical evaluations have identified some constituents from *Morinda lucida*(Table 3).

Much work needs to be done to establish scientific evidence for the efficacy claimed for Cameroon traditional medicinal plants. This work investigated the antimicrobial activity of stem bark extracts from *Alstonia boonei, Kigelia africana* and *Morinda lucida*.

Table 2. Reported constituents of *Kigelia africana*

Constituent	Reference
Isopinnatal	4
Kigelinol	4
Isokigelinol	4
Irridoids	5
Stigmasterol	16
β-sitosterol	16
Lapachol	16,17
6-methoxy-mellein	16
Kigelin	16
O-methylkigelin	16
Kigelinone	17
Kigeliol	17

Table 3. Reported constituents of *Morinda lucida*

Constituent	Reference
Carotenoids	1
Rubiadin	18
Lucidin	18
Morindone	18
Lucidin-3-β-primeveroside	18
Morindone-6-β-primeveroside	18
2-methyl-3,5,6-trihydroxyantraquinone	18
3-hydroxymorindone	18
5,6-dihydroxylucidin	18
2-methyl-3,5,6-trihydroxyantraquinone -6-β-primeveroside	18

CHAPTER III

MATERIALS AND METHODS

Plant Material

The bark from stems of mature trees of *Alstonia boonei, Kigelia africana* and *Morinda lucida* was collected in Buea, Fako Division, South West Province Cameroon (Latitude 4° 12′N, Longitude 9° 11′E). The plant material was authenticated in the Phytosanitary Department of the Ministry of Agriculture,Limbe Cameroon and shipped via air to the United States. Voucher specimens have been deposited in the Biology Department Herbarium, University of Massachusetts, Amherst, MA. Upon receipt, the plant material was stored at 5^{0} C until extracted.

Extraction

Samples of the plant material were ground separately in a blender and extracted with solvent(5 ml/g of fresh weight): methylene chloride (non polar),ethyl acetate(slightly polar), 95% ethanol(moderately polar) or acetonitrile(moderately polar). The mix of ground plant material and solvent were stored at room temperature for 24 h to allow for complete extraction. The mixture was subsequently filtered through Whatman No 1 filter paper to

remove plant debris and the filtrate was stored at 5^{0}C until used in test for antimicrobial activity.

Antimicrobial Activity

A disc diffusion susceptibility test(Barry and Thornsberry 1985) was used to determine the antimicrobial activity of the plant extracts. A yeast *Candida albicans* ATCC 14053, 2 gram positive bacterial strains,*Enterococcus faecalis* ATCC 33186 and *Staphylococcus aureus* ATCC 25923, and 2 gram negative bacterial strains, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853,were used as test organisms. All are human pathogens. Cultures of the microorganisms were prepared for inoculation by growing in Tryticase soy broth(for bacteria) or Potato dextrose broth(for yeast) for 18 h at 37^{0}C. Just before inoculation,0.5 ml of culture was diluted in 7 ml of sterile broth and used for streaking on sterile Mueller Hinton agar contained in Petri plates(9 cm in diameter). As a test system, extracts were placed in contact with the microorganisms by placing two paper discs containing extract on the agar surface. The discs were prepared by aseptically placing blank paper discs(6 mm in diameter) in clean test tubes(1 disc/tube)and covered with 1 ml of extract or pure solvent(negative control). The solvent in test tubes with extract and pure solvent was evaporated at room temperature using a nitrogen gas stream. After evaporation each disc

with extract contained about 5 mg of plant extract (difference in weight of blank paper disc, and the same paper disc containing concentrated extract). Activity of each extract against a test microorganism was compared with the activity of 2 antibiotic discs against the same organism. The antibiotic containing discs(Penicillin, Polymycin B, Methicillin, or Vancomycin, depending upon the microorganism) were placed on the agar surface in a manner similar to that used for the extract containing discs. All discs were placed on the agar surface within 5 min of inoculation with micro-organisms. The Petri plates with micro-organisms and discs were incubated for 18 h at 37^{0} C. The diameter of the microorganism free zone was measured with a metric ruler from the under side of the Petri plate.

Dose Response

To determine if a dose response existed for the extracts, a dilution series was used. In a test tube, 1 ml of the plant extract was dried under a stream of nitrogen gas leaving a 5 mg residue,which was dissolved in 1 ml of distilled water. A serial dilution was made in eight test tubes (containing 1 ml of bacterial culture each) for a concentration of extract at 0.5, 0.25,0.125, 0.0625, 0.0312, 0.0155, 0.0078 and 0.004 mg/ml respectively of the original.

After dilution the test tubes were incubated for 18 h at 37^{0} C. The cultures were poured in test tubes,and

inhibition of bacterial growth was determined by optical density measurements at 600 nm. Analysis of variance of all data was conducted using the Statistical Analysis System(SAS) (Anonymous 1988).

CHAPTER IV

RESULTS

Inhibition of Microorganism Growth by Plant Extract

Ethanol and ethyl acetate extracts of the plant material exhibited the most antimicrobial activity (Table 4). The extracts were least active against *Candida albicans*(with no zone of inhibition observed)and most active against *Staphylococcus aureus*. Extract from *Kigelia africana* had the most effective antimicrobial activity. The pattern of inhibition was dependent upon the plant species, solvent extraction and the microorganism being tested. Significant differences in the plant species were noted.

Dose Response

The bacterial inhibition observed was proportional to the concentration of the plant extract(Figure 5-13). Plant extracts at a concentration of 0.5 mg/ml produced the greatest inhibition while the least inhibition was observed at a concentration of 0.004 mg/ml. Significant differences in the concentration of the plant extracts were observed.

Highly significant differences in the effect of plant extracts on microbial inhibition, except for *Kigelia africana* extracts against *Pseudomonas aeruginosa* which were only significantly different, were observed. Differences in activity were associated with the solvents used for

extraction except for the extract of *Kigelia africana*, against *Enterococcus faecalis*.

Table 4
Inhibition of Microbial Growth by Plant Extracts

Plant extract	*C.albicans*	*E.coli*	*P.aeruginosa*	*E.faecalis*	*S.aureus*
	----------(Inhibition zone mm[1])----------				
A. boonei					
Ethyl acetate	0	0	0	0	3-6
Acetonitrile	0	0	0	0	0
Ethanol	0	0	0	0	3-4
Methylene chloride	0	0	0	0	3-4
Solvent control	0	0	0	0	0
Antibiotic 1	0	5-8	3-6	11-15	15-19
Antibiotic 2	0	5-6	3-6	4-8	7-10
M. lucida					
Ethyl acetate	0	0	3-4	3-4	4-6
Acetonitrile	0	3-4	0	0	3-5
Ethanol	0	0	3-4	3-5	4-6
Methylene chloride	0	0	0	3-4	4-6
Solvent control	0	0	0	0	0
Antibiotic 1	0	5-8	3-6	11-15	15-19
Antibiotic 2	0	5-6	3-6	4-6	7-10
K. africana					
Ethyl acetate	0	5-8	6-11	5-7	4-9
Acetonitrile	0	0	0	0	6-7
Ethanol	0	5-6	5-12	4-6	6-12
Methylene chloride	0	4-5	5-11	5-8	5-9
Solvent control	0	0	0	0	0
Antibiotic 1	0	5-8	3-6	11-15	15-19
Antibiotic 2	0	5-6	3-6	4-8	7-10

1. Minimum-maximum zones for each treatment. Each value represents the mean of three replicates.
For *E. coli*: antibiotic 1= vancomycin (30µg), antibiotic 2= polymyxin (300 units).
For *P. aeruginosa*: antibiotic 1 and antibiotic 2 = polymyxin (300 units).
For *E. faecalis*: antibiotic 1= penicillin (2 units), antibiotic 2=methicillin (5µg).
For *S. aureus*: antibiotic 1=penicillin (2 units),antibiotic 2=methicillin (5µg).
For *C. albicans*: no zones of inhibition were observed with any of the antibiotics listed above.
Significant differences between plant species were noted.

Figure 1. The effect of ethanol extract of *Kigelia africana* on *P. aeruginosa*

Figure 2. The effect of ethanol extract of *Kigelia africana* on *E. coli*

Figure 3. The effect of ethyl acetate extract of *Kigelia africana* on *E. faecalis*

Figure 4. The effect of ethanol extract of *Kigelia africana* on *S. aureus*

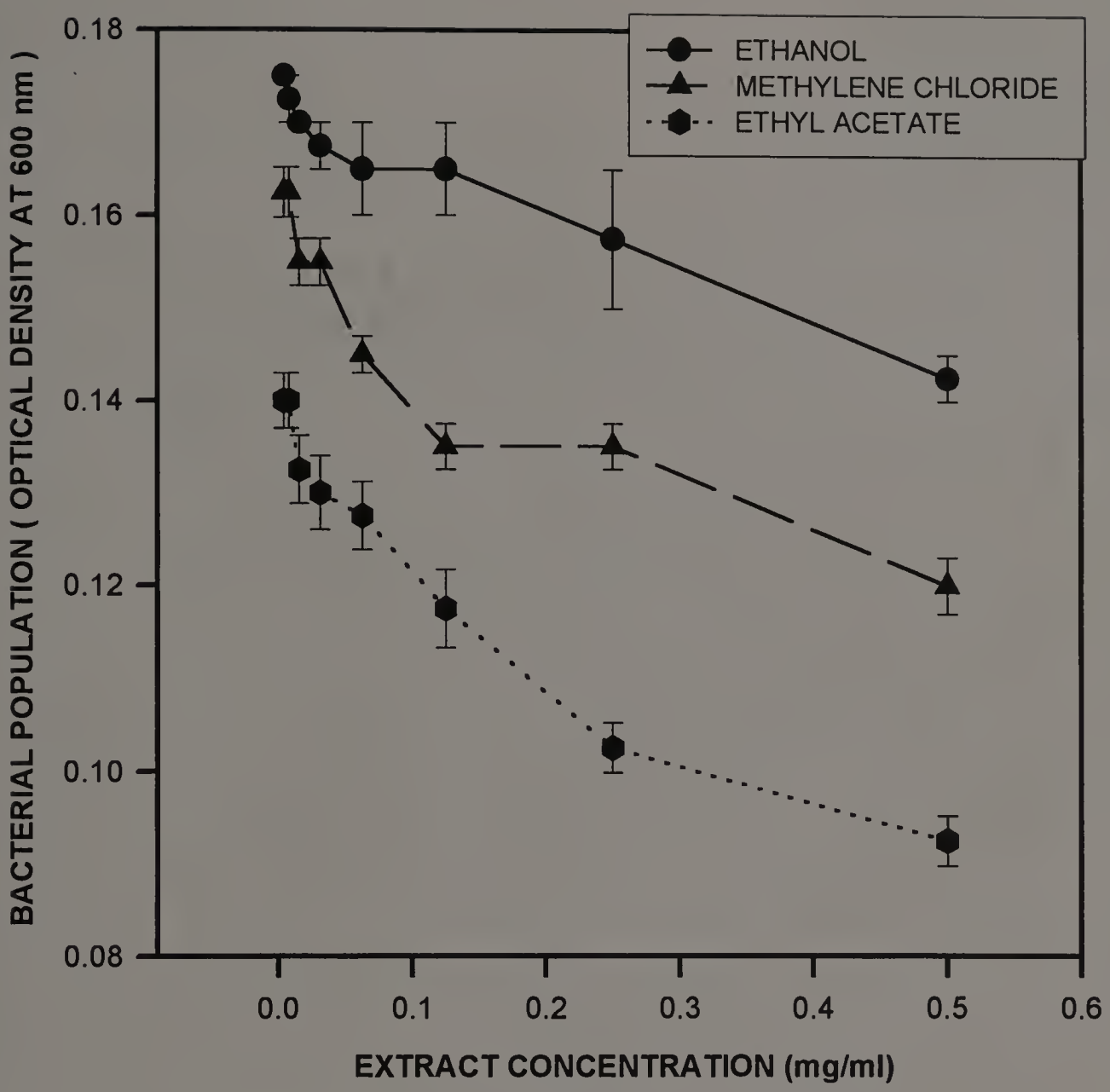

Figure 5. Effect of *Alstonia boonei* extracts against *S. aureus*.

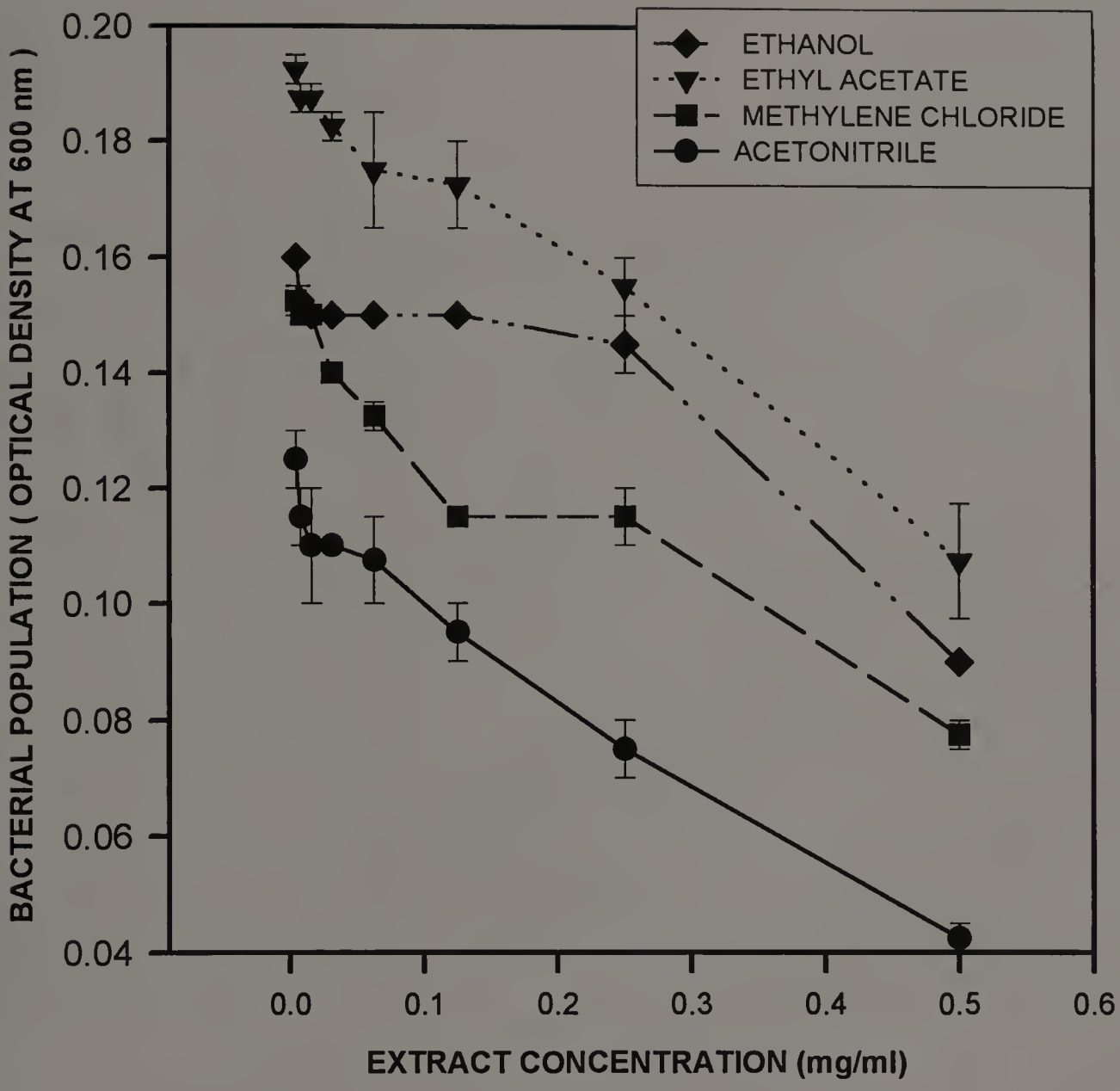

Figure 6. Effect of *Morinda lucida* extracts against *S. aureus*.

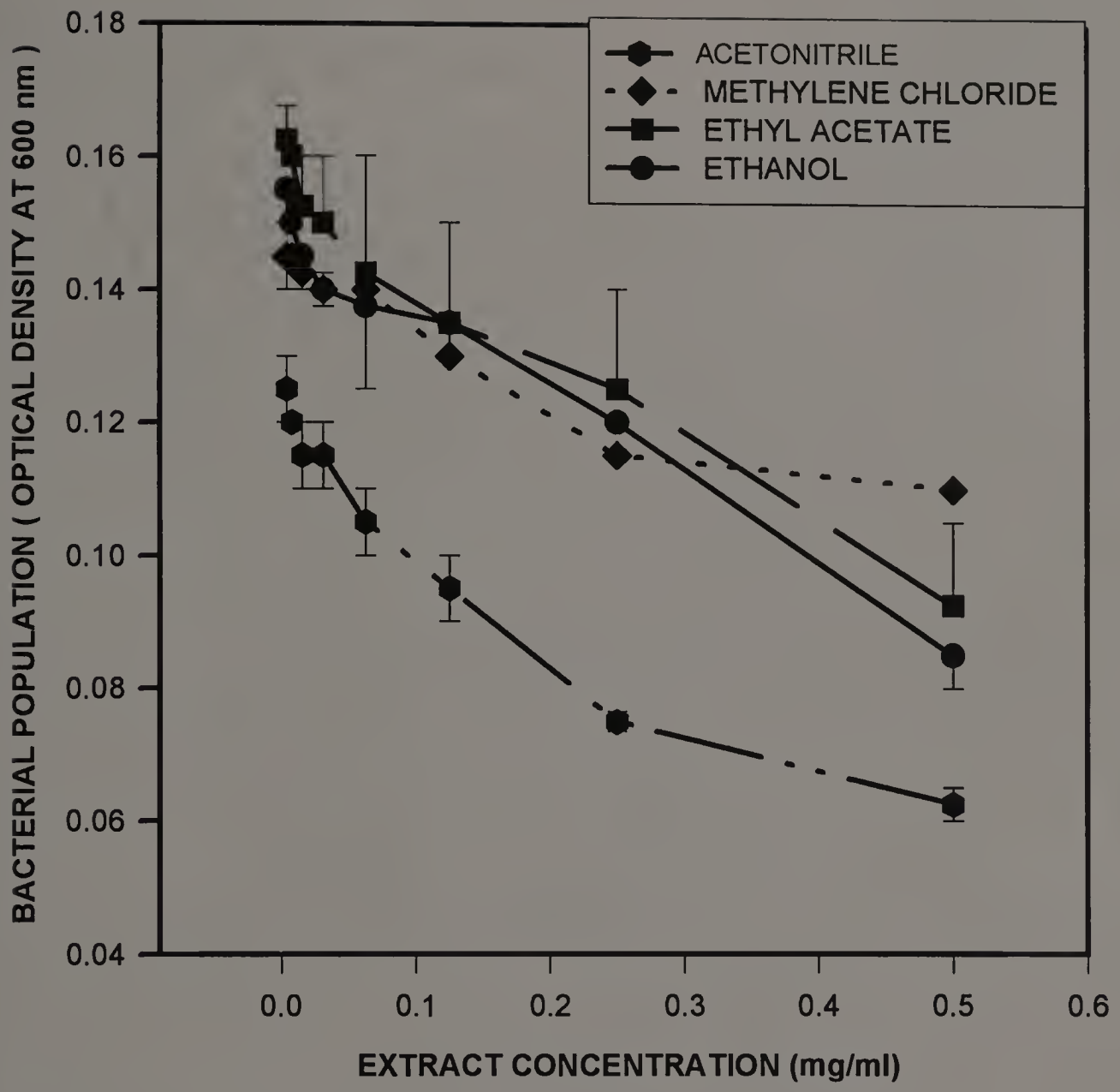

Figure 7. Effect of *Kigelia africana* extracts against *S. aureus*

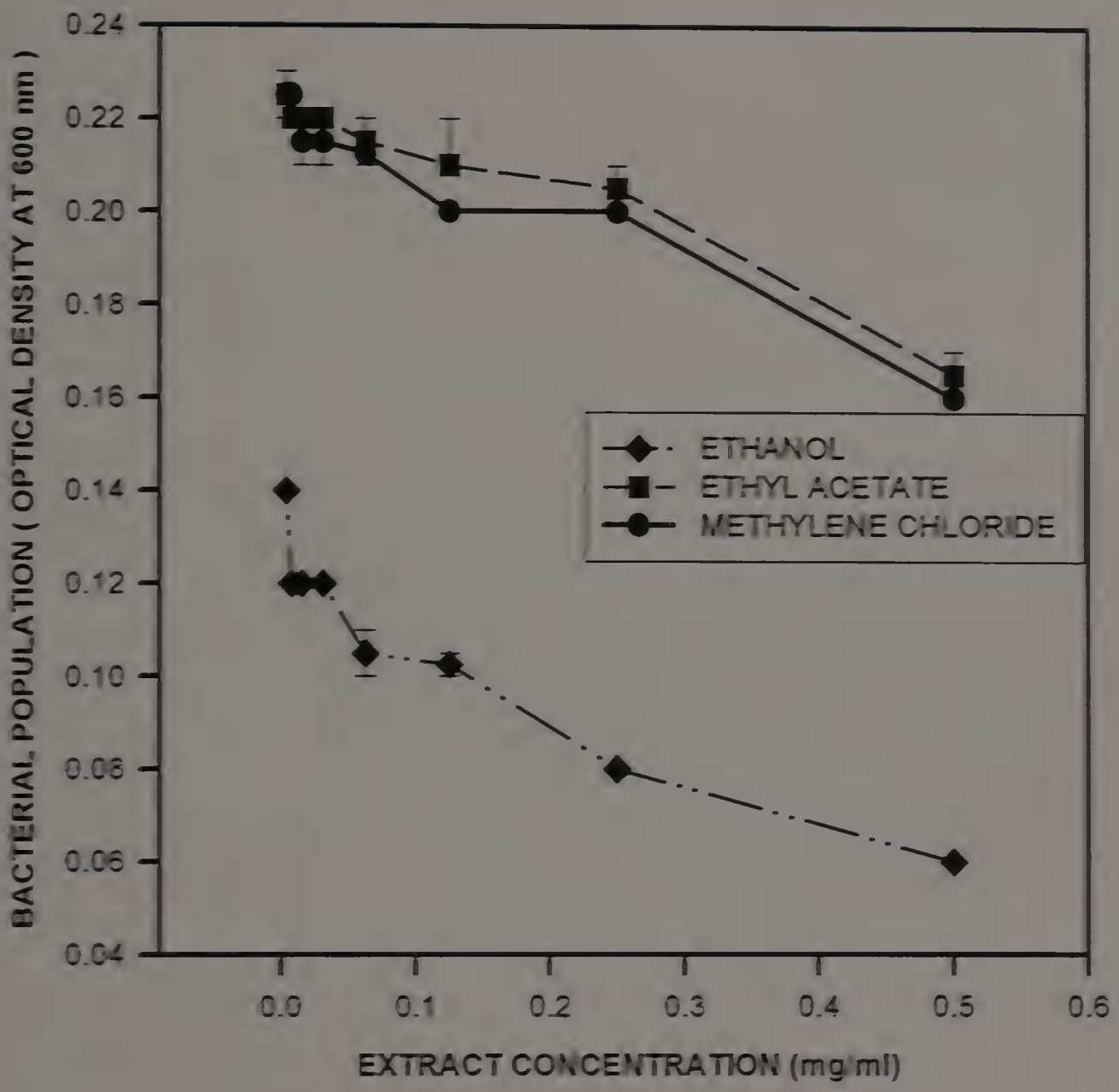

Figure 8. Effect of *Kigelia africana* extracts against *E. coli*.

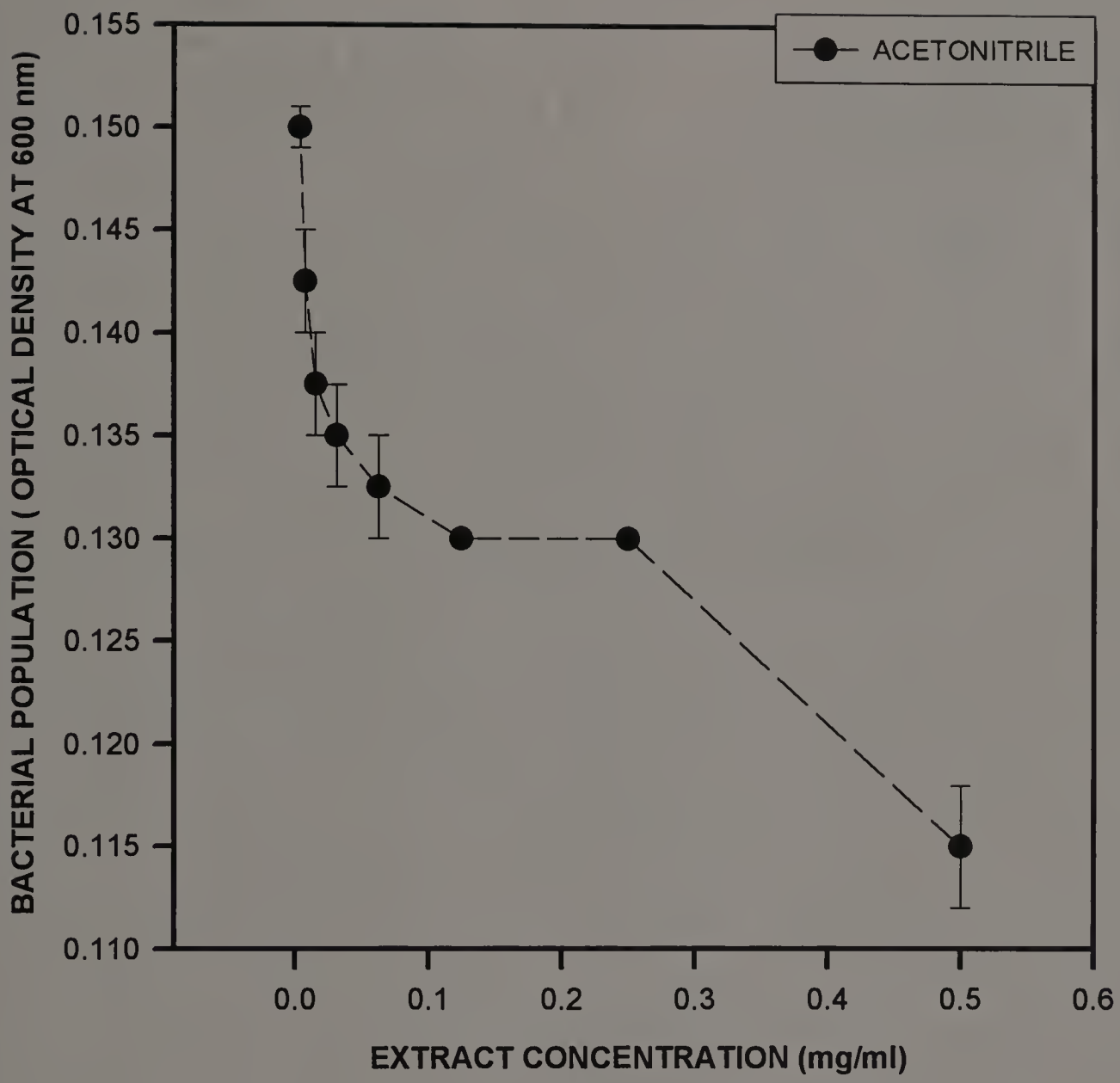

Figure 9. Effect of *Morinda lucida* extract against *E. coli.*

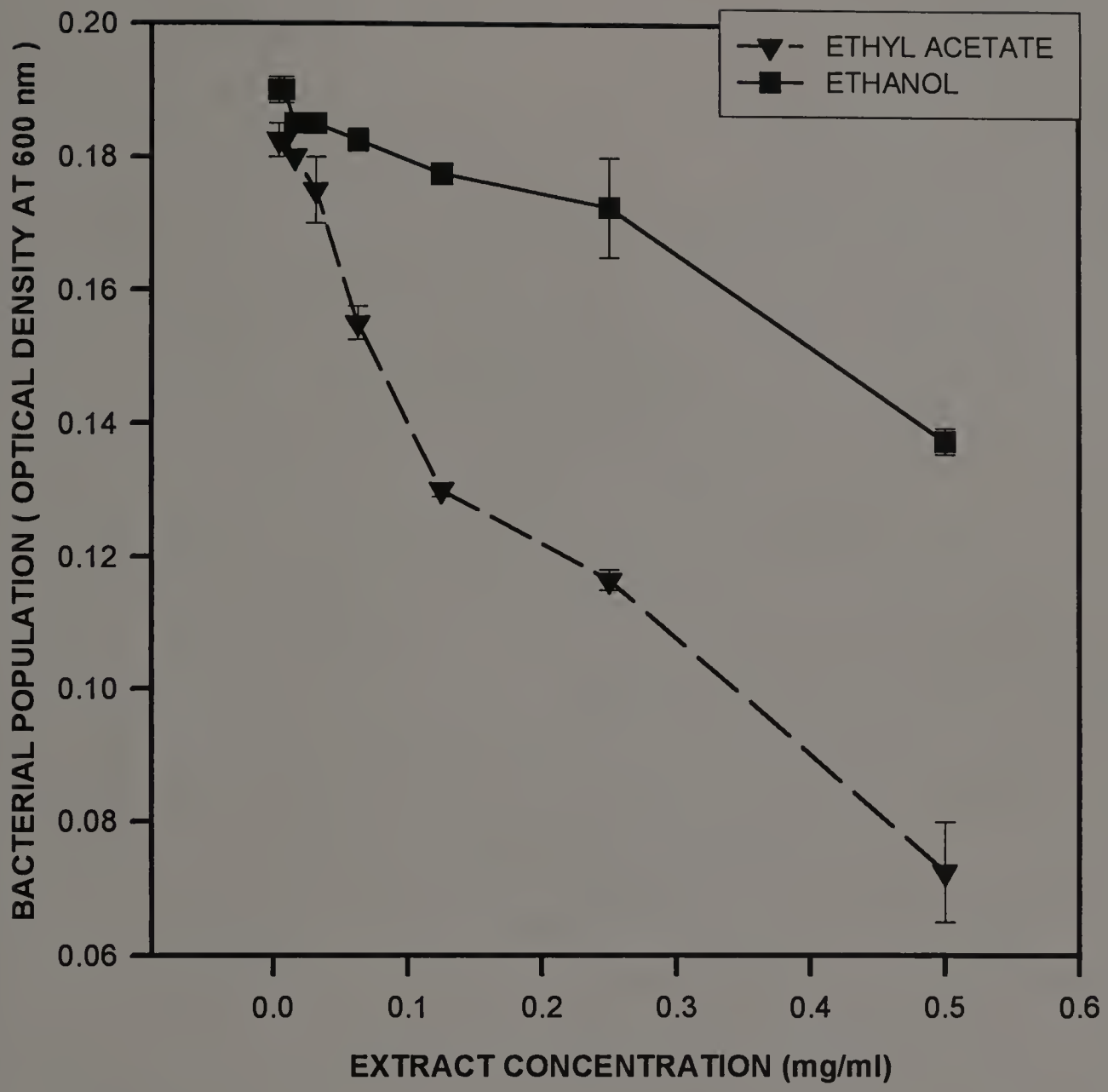

Figure 10. Effect of *Morinda lucida* extracts against *P. aeruginosa*.

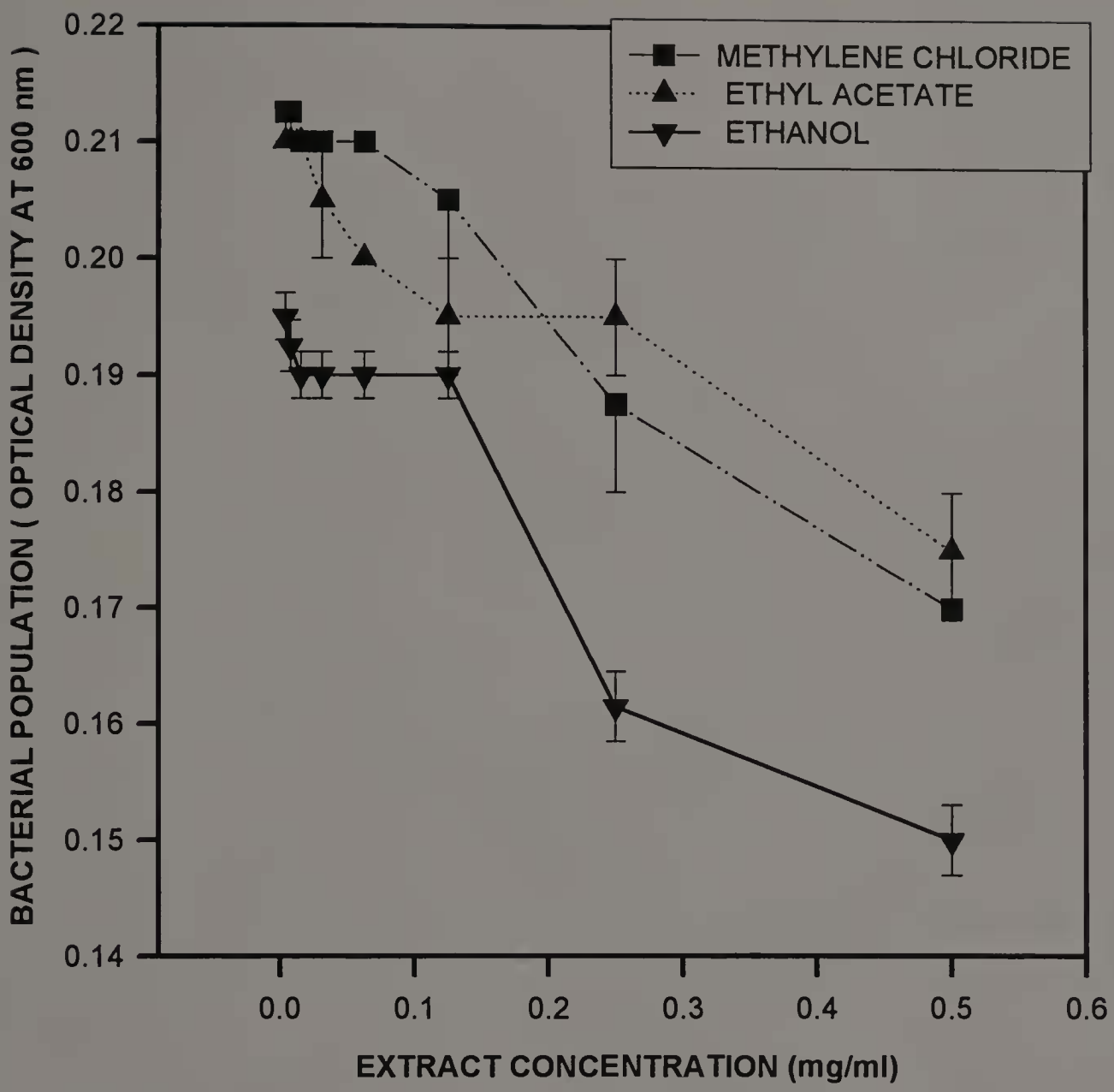

Figure 11. Effect of *Kigelia africana* extracts against *P. aeruginosa*.

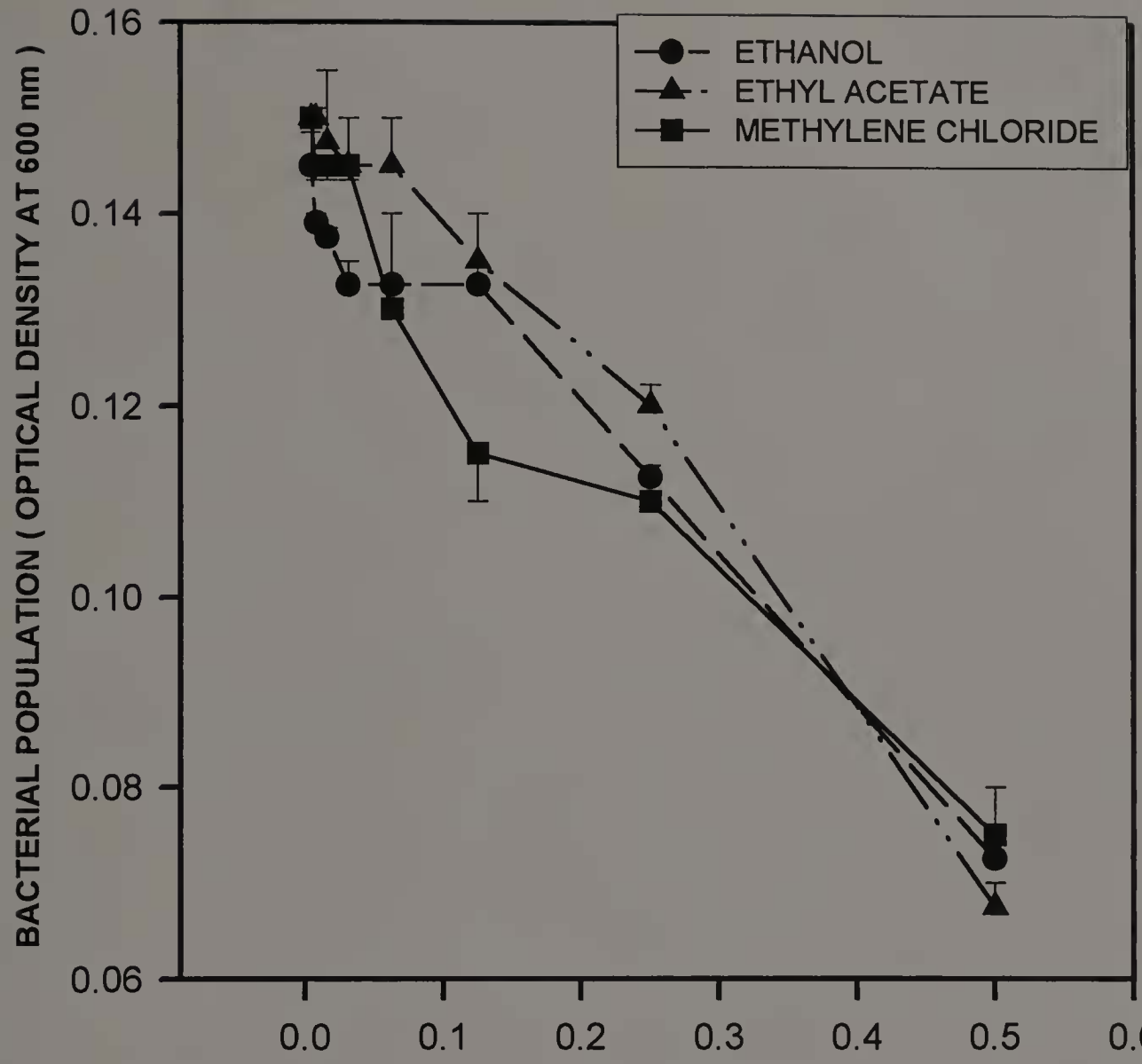

Figure 12. Effect of *Morinda lucida* extracts against *E. faecalis*.

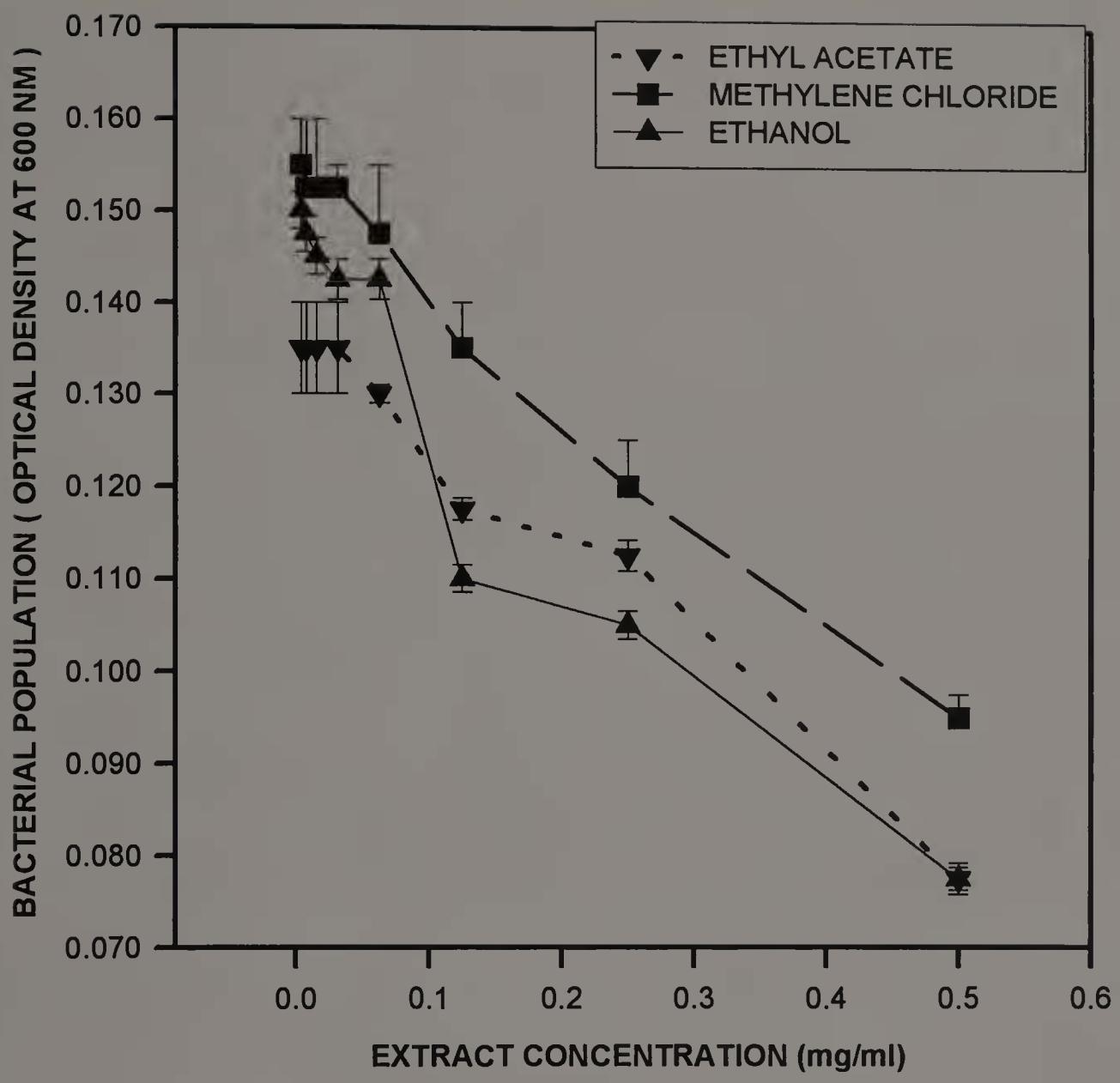

Figure 13. Effect of *Kigelia africana* extracts against *E. faecalis*.

CHAPTER V

DISCUSSION AND CONCLUSION

This study indicates that extracts from *Alstonia boonei, Kigelia africana,* and *Morinda lucida,*traditional medicinal plants from Cameroon, have some antimicrobial activity. The level of antimicrobial activity of extracts from *Kigelia africana* approached that of antibiotics, suggesting that this plant may be effective in traditional medicines and be potentially useful in other applications. The active constituents of the plant extracts were not identified.

Variation in the range of inhibition zones obtained for the disc diffusion susceptibility test is similar to the ranges for previously tested standard antibiotic control discs(Barry and Thornsberry, 1985). Extracts from *Morinda lucida* and *Kigelia africana* were active against gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*. *Kigelia africana* extracts were the most active,with the largest zone of inhibition observed with ethanol extract against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The extracts in general were more effective against gram-positive microorganisms. *Staphylococcus aureus* was the most inhibited microorganism. No inhibition was observed against *Candida albicans*. The antimicrobial activity of *Kigelia africana* extracts against *Candida albicans* reported by Akunyuli et al (1991), was not observed in this study

probably due to different methods of extraction. Akunyili et al (1991) used water, citrate, and borate buffers for extraction and the plant material was heated in a boiling water bath.

Resistance of the microorganisms to the plant extracts may be as a result of the mechanisms of microbial resistance reported by Lewis(1989). Microbial resistance in this study was probably due to destruction or inactivation of the extract by the microorganism, blockage of transport of extract, or if the extracts action is by altering a metabolic pathway, resistance may be due to a metabolic by pass in which the microbe replaces the metabolic step inhibited by the extract. A microbe may also alter the target site to eliminate or minimize binding of the extract to the target site.

The results indicates the need to use solvents of different polarities for extraction. If only one solvent was used for extraction in this study, not all the positive results recorded would have been obtain. Ethanol and ethyl acetate were the most active solvents. The failure of inhibition of microbial growth by some extracts may be due to the absence of the active constituents. The polarity of the solvent and the chemical composition might not have been ideal for isolation of the antimicrobial compound.

The plant extracts produced a significant dose-dependent activity against the bacteria tested. After a critical point, further dilution of the plant extracts

resulted in a decrease in bacterial inhibition. The bacterial inhibition observed is a scientific justification of the use of these plants in traditional medicine. The is a need for increase research on medicinal plants because of the valuable therapeutic agents they contain(Recio and Rios, 1989). Screening microorganisms for their susceptibility to antimicrobial agents is a laboratory procedure that is useful in controlling infection(Irobi and Banso, 1994).

The soaring cost of synthetic drugs and pesticides limits the availability of these products in many African countries, including Camercon thus making traditional plant medicines most important in health care. In addition, the possibility that microorganisms will continue to develop resistance to antibiotics magnifies the need to locate new sources of chemicals that could control disease organisms. Thus, a scientific evaluation on the antimicrobial activity of traditional african medicinal plants is necessary to help identify and preserve these species, especially before current populations are destroyed by deforestation.

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