

Full Length Research Paper

Antimicrobial activity of camwood (*Baphia nitida*) dyes on common human pathogens

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Antimicrobial activity of four aqueous extracts of camwood dyes obtained from different locations in Nigeria were investigated by agar diffusion, disc diffusion and agar dilution method against five clinical isolates obtained from inpatient attending the University of Port Harcourt Teaching Hospital. The isolates were *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. Broad spectrum activity revealed *B. cereus* and *S. aureus* as more susceptible with zones of inhibition ranging from 5.4 to 19.2 mm, while, *E. coli*, *P. vulgaris* and *P. aeruginosa* were the least susceptible at an inhibition zone ranging from 0.8 to 15.6 mm. Results show that the extracts exhibited inhibitory activity against the test organisms at a minimum inhibitory concentration (MIC) of 37.5 mg/ml. Phytochemical screening revealed the presence of flavonoid and a trace of alkaloids. These results show that camwood dye possessed significant antimicrobial activity and hence can be used as a remedy for pathogenic infections.

Key words: Antimicrobial activity, camwood extract, Nigeria, pathogenic microorganisms, zone of inhibition.

INTRODUCTION

The prevalence of pathogenic microorganisms that are resistant to modern antibiotics has been predominant in the past years. Diseases and their causative agents that were controlled by antibiotics before now are becoming resistant. This has led to the search for new sources of antibiotics which is of immense concern to medical practitioners (Levy and Marshall, 2004; Naveen et al., 2008). Several researchers have considered the importance of medicinal plants as reservoir of phytomedicine. These plants contain substances that can be used for therapeutic purposes. They are natural products, environmentally friendly, easily available, cheap, safer, curative and have antimicrobial properties (Egharevba and Ikhatua, 2008). Traditional medicine has remained the most affordable and easily accessible source of treatment in the health care system of certain communities while local therapy is the only means of medical treatment for some communities (Yinger and Yewhalaw, 2007). Plants have been used in traditional medicine for several years and more than 80% of the world's populace still depends

on traditional medicine for their health care needs (Idu et al., 2008). Most of the prescribed medicines in developed countries are derived from plants. Plants known for treating ailments are screened for their antimicrobial properties. Antibiotic susceptibility is used to determine the efficacy of these plants for use as antibiotics. The most basic laboratory measurement of the activity of an antimicrobial agent against an organism (MIC) and the lowest concentration of a specific antimicrobial agent that kills 99.9% of cells of a given strain of bacteria being tested (MBC) are some of the procedures used (Turnidge et al., 2003). The minimum inhibitory concentration (MIC) is a quantitative test which determines the lowest concentration of a specific antimicrobial agent needed to prevent the growth of a given organism *in vitro*. It is determined by examining the test organism ability to grow in broth cultures containing different concentrations of the antimicrobial agents. The minimum bacteriocidal concentration (MBC) is determined by assaying for organisms in those tubes from the MIC test that showed no growth, an antimicrobial agent can be bacteriostatic for one organism and bacteriocidal for another (Nester et al., 2007). These antimicrobial agents with known MIC and MBC are the drug of choice in treating infections and

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Table 1. Sources of samples collected.

Sample code	Area of collection
A	Sabon Gari Market, Kano State
B	Igbo-Ukwu Market, Anambra State
C	Idi-Araba Market, Surulere Area of Lagos State
D	Marian Market, Ejagham Area of Cross River State

A, "Majigi"; B, "Uhie"; C, "Osun"; D, "Eku"

diseases. Under laboratory conditions, efficacy testing of antimicrobial agents depends on the type of organism and its source.

In Nigeria, the local use of natural products as source of treatment cannot be overlooked due to the large number of the country's population and the inadequacy of our health care system. The country is equally very rich in medicinal plants.

A large number of these medicinal plants are used in the treatment and cure of diseases caused by microorganisms (Kamble and Deshmukh, 2008). Investigations of folk medicine have resulted in the discovery of the potential applications of medicinal plants like *Baphia nitida* lodd.

Camwood dye is a red paste obtained from *Baphia nitida* lodd, a wide spread forest plant which is commonly distributed globally especially within the coastal region of West Africa (Olowosulu and Ibrahim, 2006). Different microbial diseases are mitigated using the plant. It is applied against ringworm, stiff joints, sprains and rheumatic pains. It can equally be used for treating constipation, skin and venereal diseases (Onwukaeme, 1995; Odugbemi and Akinsulire, 2006; Onwukaeme and Lot, 2006). Various studies have been carried out to investigate the antimicrobial effects of camwood extracts. Opakunle (1988) studied the effects of camwood dye using lower concentration and found out that the dye had no antibacterial effect at that concentration. Similarly, Olowosulu and Ibrahim (2006) realized that at higher concentrations the camwood extracts elicited excellent antibacterial property.

The study was undertaken to evaluate the potentials of *B. nitida* from different locations in the country, their phytochemical composition, minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) on five clinical pathogens.

MATERIALS AND METHODS

Sample collection

Different samples of camwood dye from various local markets in Nigeria were obtained (Table 1). One hundred grams of each sample purchased from different retail outlets in each market were collected in new transparent polyethylene bags and transported to the laboratory for analyses.

Preparation of plant extract

The method of Ojo and Olufolaji (2005) was adopted. Due to the nature of the sample, aqueous cold water extraction was carried out without concentration. Fifty grams of the dried camwood samples were soaked in 125 ml distilled water for 24 h. The samples were allowed to stand for few hours, filtered through triple layered muslin cloth, and stored in the refrigerator at 4°C until it is required for use.

Test organisms

The clinical cultures used in the study were *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Proteus vulgaris* and *Pseudomonas aeruginosa* obtained from inpatients at the University of Port Harcourt Teaching Hospital. All the clinical isolates were confirmed using standard techniques in Biochemical testing of microorganisms and medical laboratory manual for tropical countries and subsequently maintained on nutrient agar at 4°C in the refrigerator (Cheesbrough, 1984, 1999).

Bacterial inoculum was prepared by inoculating a loopful of the test organism in 10 ml nutrient broth and incubated at 37°C for 24 h. One milliliter of an overnight culture of the isolates were inoculated into peptone water (standardized using sterile normal saline to obtain a population density of 10^6 cfu/ml) and used for antimicrobial susceptibility test.

Evaluation of antimicrobial activity

Various approaches were used to evaluate the antimicrobial activity of the camwood extracts.

Agar diffusion method

This method described by Osadebe and Ukwueze (2004) was adopted for the study. Broth cultures of the test isolates (0.1 ml) containing 1×10^5 cfu/ml organisms were inoculated into 20 ml of molten nutrient agar added. The content was thoroughly mixed and then allowed to solidify. Four holes were made in the plates (about 8.0 mm diameter) using a sterile cork borer and equal concentrations of the different crude camwood extracts were transferred into the holes using Pasteur pipette. The plates were allowed to stand for one hour for pre-diffusion of the extracts to occur and were incubated at 37°C for 24 h. The experiments were repeated in duplicates. After the incubation period, the plates were observed and zones of inhibition that developed were measured in millimetres.

Disc diffusion method

Using the method of Bauer et al. (1966), 5 mm filter paper disc were

Table 2. Cultural, morphological and biochemical properties of test organisms.

Parameter	Gram stain	Spore stain	Pyocyanin and fluorescent production on nutrient agar	Motility	Indole	Citrate	Urease	Catalase	Gelatin liquefaction	Coagulase	Oxidase	H ₂ S	Methyl red	Voges proskauer	Sugar fermentation				Possible microorganism
															Glucose	Lactose	Sucrose	Manitol	
Light yellow entire elevated small colonies	+Cocci	-	-	-	-	-	+	+	-	+	-	-	+	+	+	+	+	-	<i>S. aureus</i>
Small opaque circular convex smooth colonies	-Rods	-	-	+	+	-	-	-	-	+	-	-	+	-	+	+	+	-	<i>E. coli</i>
Creamy off white, circular opaque colonies with irregular edge	+Rods	+	+	+	-	+	-	+	-	-	-	-	-	+	+	-	-	+	<i>B.cereus</i>
Swarming of colonies with peritrichous flagella	-Rods	-	-	-	+	-	+	+	+	-	-	+	+	-	+	-	+	-	<i>P. vulgaris</i>
Small greenish raise smooth circular colonies	+Rods	-	+	+	-	-	-	+	+	-	+	-	-	-	+	-	-	+	<i>P. aeruginosa</i>

+, Positive; -, negative.

soaked with 0.1 ml of the different camwood extracts, placed on pre-inoculated agar, kept at 4°C for 30 min for pre-diffusion and incubated at 37°C for 24 h. The results were recorded in terms of zones of inhibition.

Agar dilution method

The agar dilution method was used to determine the minimum inhibitory concentration of the camwood extracts which exerted the greatest inhibitory activity against the test organisms. Ten milliliters of different double strength concentration of the extracts (150, 75, 37.5, 18.75, 9.38, 4.69, 2.34, 1.17 and 0.59) mg/ml was mixed with 10 ml of double strength agar media, poured into sterile Petri dish and were allowed to set. After which 0.1 ml of the overnight broth cultures of the test organisms were streaked on the

plates, incubated at appropriate conditions, as stated under agar diffusion test, after which the presence or absence of growth was noted. The MIC was taken as the lowest concentration of the extract that did not permit any visible. MBC was determined by transferring inocula from tubes showing no detectable growth (no turbidity) after 24 h incubation onto nutrient agar and incubated further at 37°C for 24 h to determine the minimum bacteriocidal concentration of the dye required to kill the organism. The plates of the extract that showed no growth indicated as bacteriocidal was recorded as the MBC.

Phytochemical screening

The method of Brian and Turner (1975) and Trease and

Evans (2002) were used for screening the major constituents of the various camwood dyes from different location in Nigeria. Trease and Evans (2002) were used for the presence of saponin, alkaloids and tannins. Shinoda test for flavenoids and Buchard test was used for steroids.

RESULTS

The antimicrobial activity of the various camwood dyes were examined using five clinical isolates (*S. aureus*, *E. coli*, *B. cereus*, *P. vulgaris* and *P. aeruginosa*) which were properly identified as can be seen from Table 2. The antibacterial activity of crude camwood dyes extracts to the test

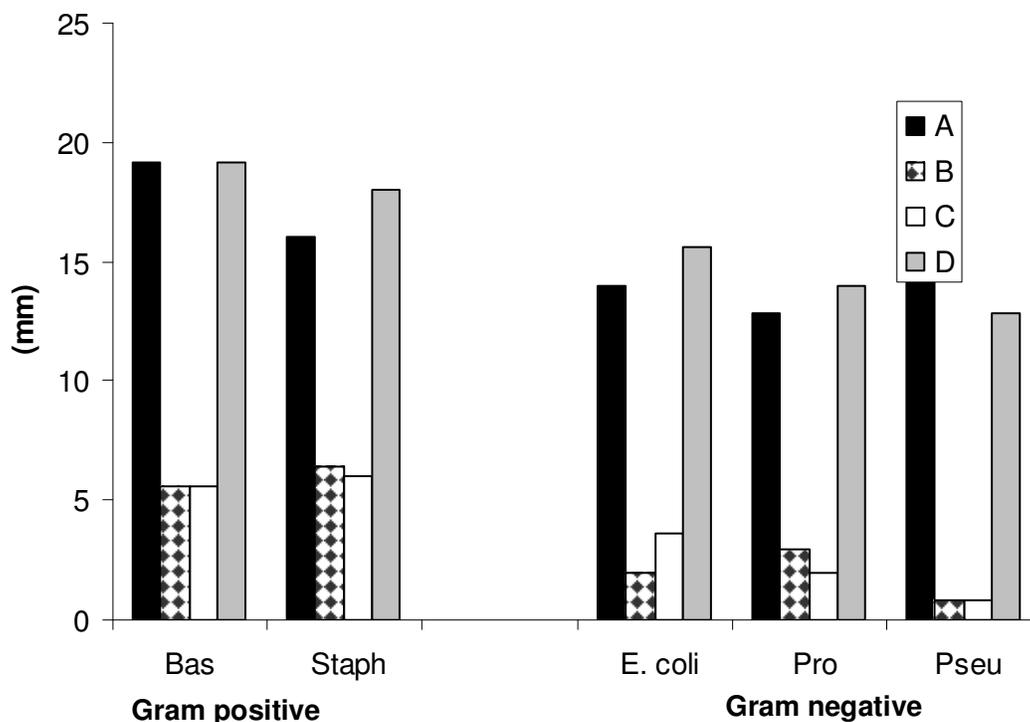


Figure 1. Antibacterial activity of crude camwood dye extracts against the test organisms by agar diffusion method. See Table 1 for samples A to D.

organisms are shown in Figures 1 and 2. The results show that the dyes exerted greater inhibitory activity against the Gram positive organism. Among all the dyes sampled, Ekui dye exerted the greatest inhibitory activity, followed by Majigi dye and the least were Uhie and Osun dyes respectively. Comparison of the zones of inhibition revealed very slight differences, *B. cereus* predominated at 19.20 and 18.60 mm zones of inhibition, *S. aureus* at 18.00 and 16.3 mm respectively; *E. coli* (15.60 and 14.00 mm), *P. vulgaris* (14.00 and 12.2 mm) and *P. aeruginosa* (12.80 and 10.40 mm). The MIC and MBC values of the extracts are shown in Figures 3 and 4 respectively. Majigi dye exerted the highest activity against two organisms *B. cereus* and *E. coli* at 37.5 mg/ml with the lowest activity at 75 mg/ml against *S. aureus*, *P. vulgaris* and *P. aeruginosa*. Uhie dye showed the highest activity against three organisms at 37.5 mg/ml *S. aureus*, *E. coli*, and *P. vulgaris* while the lowest activity of 75 mg/ml was showed against *B. cereus* and *P. aeruginosa*. Osun dye at 37.5 mg/ml exhibited activity against *S. aureus* and *P. vulgaris* but at 75 mg/ml had the least activity against *B. cereus*, *E. coli* and *P. aeruginosa*. The highest activity of 37.5 mg/ml was revealed against *B. cereus* and *P. aeruginosa* by Ekui dye with the least activity of 75 mg/ml against *S. aureus*, *E. coli* and *P. vulgaris*. Table 3 represents the secondary metabolites present in the various camwood dyes. Flavonoid was prominent, followed by alkaloid but the others were absent.

DISCUSSION

The sensitivity of test microorganisms against aqueous extracts of various camwood dyes from different locations in Nigeria was studied. Broad spectrum activities of both Gram positive and Gram negative microorganisms revealed *B. cereus* and *S. aureus* as more susceptible to the inhibitory activities of various camwood dyes, compared to *P. aeruginosa*, *E. coli* and *P. vulgaris*. Olowosulu and Ibrahim (2006) obtained similar results with extracts of *B. nitida*. Several workers throughout the world working on potential applications of medical plants like *Ocimum sanctum* extracts were in conformity with the results obtained (Prakash, 2006; Kamble and Deshmukh, 2008). This may be due to the resistance pattern and mechanism of bacterial strains which is of utmost importance in view of the necessity for the worldwide study of antibacterial resistance which has recently been associated with major disease outbreaks (Singleton, 1999; Lateef et al., 2004). The level of antimicrobial agent's resistance reflects on the misuse and abuse of the antibiotics in the environment. Consequently, most antibiotics are given in hospital without clear evidence or adequate medical instructions. Most of the time, toxic broad-spectrum antibiotics are given in place of narrow-spectrum drugs before culturing and sensitivity testing, placing the patient at a very high risk of side effects, super infections and the detection of drug resistant

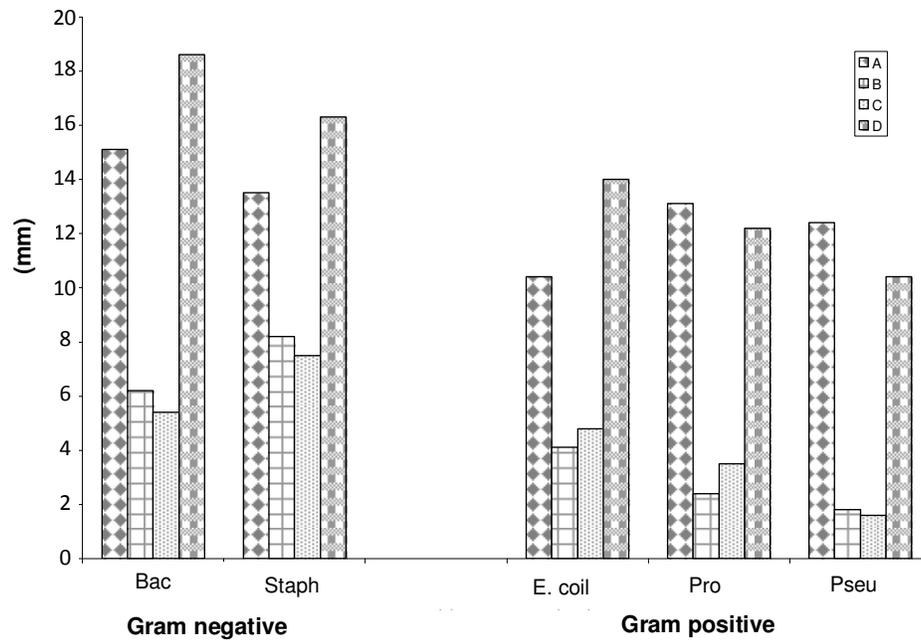


Figure 2. Antibacterial activity of crude camwood dyes extracts against the test organisms using disc diffusion method. See Table 1 for samples A to D.

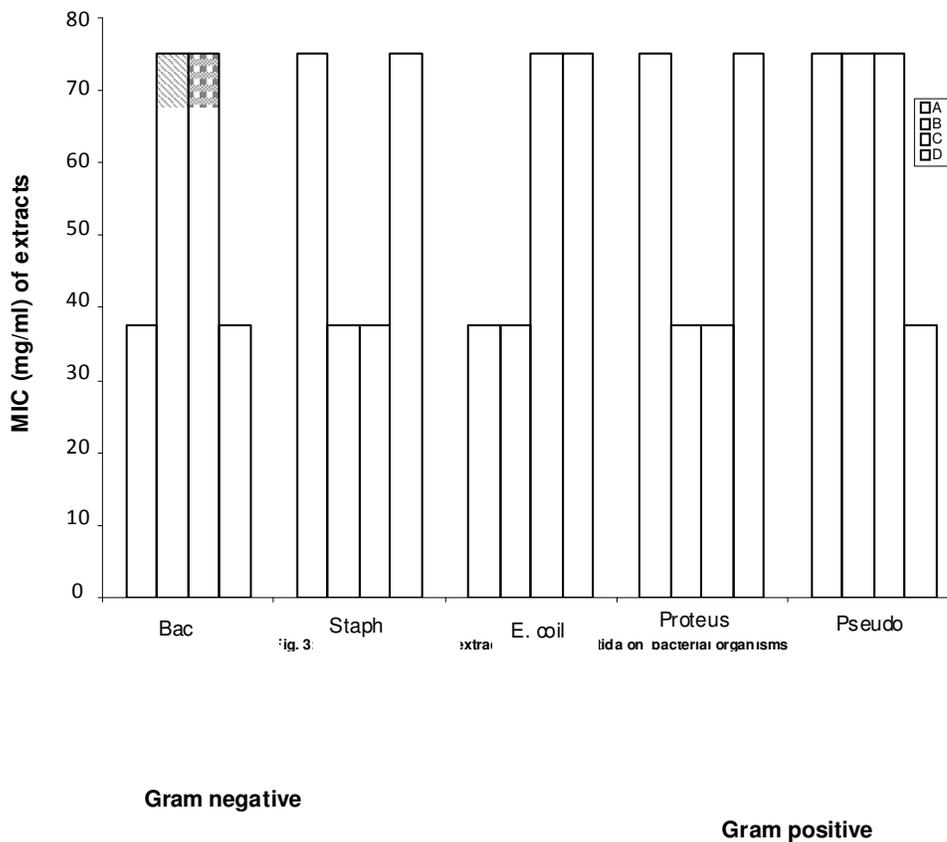


Figure 3. MIC of the different extracts of *Baphia nitida* on bacterial organisms. See Table 1 for samples A to D.

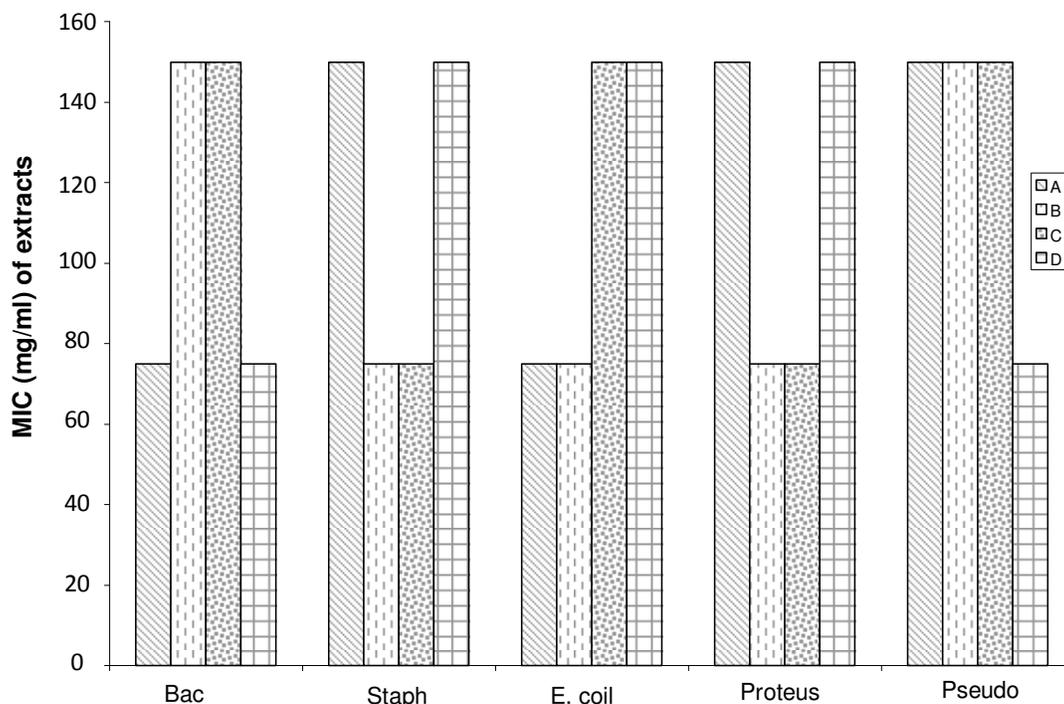


Figure 4. MBC of the different extracts of *Baphia nitida* on bacterial organisms. See Table 1 for samples A to D.

Table 3. Phytochemical profile of the different extracts of *Baphia nitida*.

Chemical constituent	<i>Baphia nitida</i> extract			
	A	B	C	D
Alkaloids	+	+/-	+/-	+
Tannins	-	-	-	-
Saponins	-	-	-	-
Flavonoids	++	++	++	++
Steroids	-	-	-	-

++ = Strongly positive, +/- = weakly positive, + = positive, - = negative. See Table 1 for samples A to D.

mutants (Singleton, 1999; Lateef et al., 2004). The MIC showed higher activity against *B. cereus*, *S. aureus*, *E. coli*, *P. vulgaris*, while *P. aeruginosa* had low activity. The presence of flavonoid with a trace of alkaloid shows that these secondary metabolites are responsible for the antimicrobial activity exhibited by this dyes (Olowosulu and Ibrahim, 2006). Flavonoids are groups of phenolic compounds from plants that have the ability to complex with extracellular, soluble proteins and lipoflavonoids in disrupting the microbial cell membrane and exert antimicrobial property (Boris, 1996; Tsuchiya et al., 1996).

This is of great significance in the health care delivery system as the dyes can be used as an alternative to medicine in the treatment of infectious diseases, thus

reducing the cost of the drugs to the affected individuals (Ogueke et al., 2006). Most of these dyes are used to cure infectious diseases relating to the skin, urinary tract, enteritis and other gastrointestinal problems which the test isolates are associated with. All the camwood dyes used in this study exhibited a level of antibacterial activity and the dyes act differently on different microorganisms. The difference in sensitivity can be due to the different growth rate of microorganisms, size, temperature, inoculum and the test method adopted (Gail, and Jon, 1995).

The zones of inhibition of camwood dyes (Figures 1 and 2) indicated that the extracts, have good antimicrobial activity even in crude form, the inhibition zones are clearly indicative of good antibacterial action. All the

camwood dyes used in the study showed different level of antibacterial activity on different microorganisms. The results show that the dye possesses some level of antimicrobial activity and can be used as a remedy for pathogenic infections.

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