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Antimicrobial Potency of Chloroform, Acetone and Ethyl Acetate Extracts of Home Spider Cobwebs

[1] Anukam, U. S. , [1]Ndu-Osuoji, I. C. , [2]Kamalu, N. A., [3]Nwoko, O.E.and [3]Mmasi U.G.

1 Microbiology/Biochemistry department, Federal polytechnic Nekede, Owerri Imo State, Nigeria.

2 Animal and Environmental Biology department, Kingsley Ozumba Mbadiwe University Ideato, Imo State Nigeria.

3. Biology Education Department Alvan Ikoku Federal University of Education Owerri, Imo State Nigeria.

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ABSTRACT

This study investigated the antimicrobial potency of chloroform, acetone and ethyl acetate extracts of home spider cobwebs. Cobwebs used in this study were obtained from different homes using a stick to collect the webs. The different solvents were used in extraction of the cobwebs using soaking method. Standard microbiological methods were adopted in the determination of the antimicrobial activities of the different extracts against; Staphylococcus, Streptococcus, Klebsiella, Pseudomonas and Aspergillus species. Zones of inhibition of the extracts were determined using agar well diffusion technique. Antimicrobial susceptible testing of the cobweb extracts against the test organisms revealed zones of inhibition; 17mm to 20mm with ethyl acetate extract, 13mm to 16mm with acetone extract and none with chloroform extract. The extracts showed zones of inhibition only on Staphylococcus and Pseudomonas species. Tube dilution technique was adopted in the determination of the minimum inhibitory concentration (MIC) of the extracts against the test organisms. Inhibitory effect was recorded at 250mg/ml, 500mg/ml with ethyl acetate extract and 500mg/ml with acetone extract. Minimum bactericidal concentration (MBC) revealed that the extracts showed bactericidal effect at 500mg/ml. Ethyl acetate showed better antimicrobial activity compared to other extracts. Thus, isolation and purification of cobweb extracts could result in better antimicrobial activities.

Keywords: Antimicrobials, Cobwebs and solvents.

I. INTRODUCTION

Spiders are diverse collection of animals, taxonomically grouped within the order Araneae, which in total contains more than 40,000 species across 109 families (Platnick, 2011). Spiders also universally possess eight legs (provided they are healthy) either six or eight eyes and spinnerets for extruding silk. Spider web" is typically used to refer to a web that is apparently still in use (i.e. clean), whereas "cobweb" refers to abandoned (i.e. dusty) webs (David et al., 2011). However, "cobweb" is used to describe the tangled three-dimensional web of some spiders of the Theridiidae family. Whilst this large family is also known as the tangle-web spiders, cobweb spiders and comb-footed spiders, they actually have a huge range of web architectures.

Several different types of silk may be used in web construction, including a "sticky" capture silk and "fluffy" capture silk, depending on the type of spider. Webs may be in a vertical plane (most orb webs), a horizontal plane (sheet webs), or at any angle in between. It is hypothesized that these types of aerial webs co-evolved with the evolution of winged insects. As insects are spiders' main prey, it is likely that they would impose strong selectional forces on the foraging behavior of spiders (Upghade and Deshmukh, 2019).

Most commonly found in the sheet-web spider families, some webs will have loose, irregular tangles of silk above them. These tangled obstacle courses serve to disorient and knock down flying insects, making them more vulnerable to being trapped on the web below. They may also help to protect the spider from predators such as birds and wasps. Some species of spider do not use webs for capturing prey directly, instead pouncing from concealment (e.g. trapdoor spiders) or running them down in open chase (e.g. wolf spiders) (Simon and Goodacre, 2012).thus the need to determine the antimicrobial potency of chloroform, acetone And ethyl acetate extracts of home Spider cobwebs

II. MATERIALS AND METHODS

Collection of cobweb

Cobwebs used in this study were obtained from different homes using a stick to collect the webs. The webs were taken to the laboratory and washed thoroughly using distilled water to remove dust and other extraneous materials. The washed web was allowed to air-dry at room temperature (30 ± 2 ºC) and kept in air-tight container until further use.

Sterilization of glasswares and media

All the glasswares used in this study were sterilized using laboratory hot air oven at temperature of 160 ^{0}C for 1 hour and media (Mueller-Hinton agar, nutrient agar, sabouraud dextrose agar and peptone water) used in this study were sterilized using the autoclave at a temperature of 121^{0} C at 15psi for 15 minutes. After the sterilization, the media were brought out together with the glassware and kept on a clean laboratory bench. The media were poured into the Petri dishes when cooled to 45 ^{O}C and allowed to solidify (Cheesbrough, 2010).

Bacterial isolates used for the antimicrobial activities

The bacterial isolates used for this study were; Klebsiella, Pseudomonas, Staphylococcus, Streptococcus and Aspergillus species. They were isolated from clinical samples from human.

Preparation and extraction of spider web

The method described by Mirghani et al. (2012) was adopted with slight modification for the preparation and extraction of cobwebs. Ten (10) grams of spider web was weighed and hydrolyzed with 90 ml of ethyl acetate, chloroform and acetone, respectively to extract sample for one week. The hydrolyzed cobwebs were centrifuged at 4000 rpm for 10 minutes. The supernatant obtained was collected and labeled as spider web extract.

Standardization of Inocula

Standard inocula of 0.5 M McFarland's standard solution of the test organisms were used for the study as described by CLSI (2010).

Determination of antimicrobial activity of the cobweb extracts

The antibacterial activity of cobweb extracts was tested by standard agar well diffusion method as described by Al-Kalifawi and Kadem (2017). Wells were made using sterile cork-borer under aseptic conditions. The inocula were prepared by diluting the overnight cultures with peptone water to 0.5 McFarland standards and were swabbed onto the plate. Cobweb extracts was loaded on marked wells with the help of micropipette under aseptic conditions and incubated at 37 ºC for 24 hours. The zone of inhibition was measured and expressed in millimeters.

Test for minimum inhibitory concentration (MIC)

For the minimum inhibitory concentration, one millilitre (1ml) of the cobweb extract was dissolved in two millilitres (2ml) of peptone water separately. This gives 500mg/ml. Thereafter, two fold serial dilutions was carried out from the 500 mg/ml concentration by transferring 1 ml of the 500 mg/ml concentration to 2 ml of peptone water contained in a test tube and homogenized properly. This procedure of transferring 1 ml of the tube to 2 ml of peptone water contained in the subsequent tube was continued until the eighth tube. The following concentrations were thereafter obtained: 500mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.65 mg/ml. Three drops of overnight broth cultures of the test organisms were each inoculated into the dilutions. The tubes were then incubated at 37^{0}C for 24 hours. The lowest concentration of each of the spider web extract that inhibited the growth of the test organisms were recorded as the MIC.

Test for minimum bactericidal concentration

Tubes showing no visible growth from the MIC test were sub cultured onto sterile nutrient agar plates. The plates were incubated at 37^{0}C for 24 hours for the bacterial. The lowest concentration of the spider web extract yielding no growth or showed on growth was recorded as the Minimum Bactericidal Concentration.

III. RESULTS

Table 1 showed the antimicrobial susceptible testing of the cobweb extracts against the test organisms. The zones of inhibition recorded ranged from 14mm to 26mm with ethyl acetate extract, 16mm to 20mm with acetone extract and 10mm to 18mm with chloroform extract. The extracts showed greater zones of inhibition against Staphylococcus and Pseudomonas species.

Table .2 showed the result of the minimum inhibitory concentration (MIC) of the

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extracts against the test organisms. Inhibitory effect was recorded at 125mg/ml to 250mg/ml with ethyl acetate extract, 250mg/ml with acetone extract and 250mg/ml to 500mg/ml with chloroform extract.

Table .3 showed the result of the minimum bactericidal concentration (MBC) of the extracts against the test organisms. Results revealed that the extracts showed bacteriocidal effect at 500mg/ml. Ethyl acetate showed better antimicrobial activity compared to other extracts

Tab1e 1 :Antimicrobial susceptibility testing of the cobweb extracts

Solvents/zones of inhibition (mm)				
Test organisms	**Ethyl acetate**	**Acetone**	**Chloroform**	**CLSI Guideline**
Pseudomonas species	21	16	18	R= Resistant (0-12mm)
Streptococcus species	19	18	16	S= Susceptible (16mm and above)
Staphylococcus species	26	18	18	
Klebsiella species	26	20	16	
Aspergillus species	14	16	10	

Key: mm = Millimeter CLSI = Clinical Laboratory Standard Institute
NI = No inhibition

Table 2:Minimum inhibitory concentration (MIC) of the cobweb extracts

Solvents/concentrations (mg/ml)			
Test organisms	**Ethyl acetate**	**Acetone**	**Chloroform**
Pseudomonas species	125	250	250
Streptococcus species	250	250	ND
Staphylococcus species	125	250	250
Klebsiella species	125	250	500

Key: mg/ml = Milligram per milliliter ND = Not determined

Table 3 Minimum bactericidal concentrations of the cobweb extracts

Solvents/concentrations (mg/ml)			
Test organisms	**Ethyl acetate**	**Acetone**	**Chloroform**
Pseudomonas species	500	500	ND
Streptococcus species	500	ND	ND
Staphylococcus species	500	ND	500
Klebsiella species	500	500	ND

Key: mg/ml = Milligram per milliliter ND = Not determined

IV. DISCUSSION

The unique structural diversity inherent in natural products continues to be recognized for its value in the drug discovery process (Iwai et al., 2009). This study investigated the antimicrobial of chloroform, acetone and ethyl acetate extracts of home spider cobwebs. The results of this study are shown in Table 4.1 to Table 4.3 above.

Table 1 showed the antimicrobial susceptible testing of the cobweb extracts against the test organisms. The zones of inhibition recorded ranged from 14mm to 26mm with ethyl acetate extract, 16mm to 20mm with acetone extract and 10mm to 18mm with chloroform extract. The extracts showed greater zones of inhibition against Staphylococcus and Klebsiella species. From the results, ethyl acetate and acetone showed antimicrobial activities against the test organisms. Chloroform extract showed lesser zones of inhibition compare to other solvents. This could be as a result of the solubility of the cobweb in each of the solvents.

Iqbal et al. (2019), in their study on antimicrobial activity of extracts of spider web silk showed that different extracts showed zones of inhibition ranging from 6mm to 20mm. Maximum antimicrobial activity was recorded with chloroform and acetone followed by ethyl acetate with S. aureus being the most susceptible organism. Al-Kalifawi and Kadem (2017) reported that the acetone extract had effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition ranging from 12 mm to 16mm. The results of this study are in line with their report.

Table 2 showed the result of the minimum inhibitory concentration (MIC) of the extracts against the test organisms. Inhibitory effect was recorded at 125mg/ml to 250mg/ml with ethyl acetate extract, 250mg/ml with acetone extract and 250mg/ml to 500mg/ml with chloroform extract. Ulhaq et al. (2019) reported minimum inhibitory effect of spider web against multi-drug resistant E. coli, Salmonella typhi, Bacillus species, Streptococcus pneumoniae and Enterococcus faecalis to range from 3.3 mg/ml to 11.55mg/ml.

Table .3 showed the result of the minimum bactericidal concentration (MBC) of the extracts against the test organisms. Results revealed that the extracts showed bacteriocidal effect at 500mg/ml. Ethyl acetate showed better antimicrobial activity compared to other extracts. Comparing the results obtained in this study with other researchers, it could be concluded that not all cobwebs have antimicrobial properties. Similarly, type of solvent has effect on the extraction of active ingredients needed for antimicrobial activity. This is in agreement with the report of Angela and Derek (2020).

V. CONCLUSION

The results from this finding showed that cobwebs have antimicrobial agents that are capable of inhibiting pathogenic microorganisms. From the results, different solvents showed different extracting efficacy. Ethyl acetate and acetone showed zones of inhibition against Staphylococcus and Klebsiella species. However, the extracts showed bacteriostatic effects against these two organisms. Isolation and purification of cobweb extracts could result in better antimicrobial activities.

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