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Full Length Research Paper

Evaluation of the effect of colloidal silver on the antibacterial activity of ethanolic extract of the lichen *Parmelia Perlata*

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Substances extracted from lichens have previously been reported to possess antimicrobial activities against various groups of bacteria, fungi and viruses. Due to the high abundance of *Parmelia perlata* in the Eastern parts of Nigeria, we decided to explore the effect of colloidal silver on the antibacterial activity against *Staphylococcus aureus*. The lichen extract was obtained via cold maceration with ethanol. The phytochemical tests was carried out on the extract of lichen. The minimum inhibitory zone MICs of the ethanolic extract against *S. aureus* was determine and the solution of the colloidal silver was also determine separately on *S. auerus*, the MIC of each was noted and their admixture in the presence of concentration of colloidal silver using bore- plate method. Result of the phytochemical tests showed the presence of flavonoids, saponins, tannins, glycosides, steroidal aglycone, carbohydrates. The result also shows that, the ethanol extract of the lichen has activity on *S. aureus* while the colloidal silver at the dilution used, has very little activity on the *S. aureus*. It was also seen from the result that the zone of inhibitory of the ethanol extract was increased in the presence of colloidal silver and MICs is concentration dependent. It was therefore finally concluded that colloidal silver potentates the effect of ethanol extract of the lichen *P. perlata*

Key words: *Parmelia perlata*, lichen, *Staphylococcus aureus*, evaluation, colloidal silver.

INTRODUCTION

Over the centuries, plants have served man as a source of drugs for the treatment of microbial infections. Quinine, artemisinin and several other drugs are examples of plant–derived chemotherapeutic agents which have been successfully used in the treatment of various health cases of man. Virtually all phyla (including lichens) from thallophytes to the higher phyla have come under investtigations. Lichens are symbiotic association between an alga and a fungus. Thus, they develop with a unique morphological form different entirely from either partner. This composite form generally has separate chemical and physiological properties (Trease et al., 2002). The fungi provide strength, shelter and reproduction while the algae provide food by photosynthesis. Lichens are known to have antimicrobial activity (Smith, 1962). The compounds responsible for this activity are polysaccharides, depsides, benzofuran derivatives or fatty acid derivatives (Berdy, 1982). In Europe, *Parmelia perlata* has been used as light brown dye for wool as well as bio-indicator of air pollution of heavy metals such as zinc, lead, cadmium copper and mercury (Pilegaard., 1978). *P. perlata* contain acidic substance that has been used as an antibiotic in several countries as a topical antibacterial agent for human skin diseases (Ketchum, 1984). Hence this study is to evaluate the effect of colloidal silver on the antimicrobial effect of ethanol extract of lichen

MATERIALS AND METHODS

The materials used in performing the experiment include: colloidal silver concentrate (Formor international, USA) Dimethylsulphoxide (DMSO). The culture media is nutrient agar (Oxoid), 95% ethanol; distilled water.

Collection of the plant material

The lichens were collected from palm tree and dead tree trunks in Nsukka LGA, Enugu state; Nigeria by Mr Ogboso Kalu of the de-

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partment of pharmaceutics, university of Nigeria, Nsukka It was identified in the department of botany by the garden staff and a Voucher specimen has been deposited in university harberium. The aerial and thallus portion of the plant material were air dried for 72 h, and ground to a powdered form using a hand milling. The lichen powder was then stored in a corked conical flask to be used for experiments.

Test organism

The test microorganisms used for this research was clinical isolate of *S. aureus.* This was obtained from the department of pharmaceutics, university of Nigeria, Nsukka and stored on the nutrient agar slants.

Extraction procedure

The powdered plant material (200 g) was extracted by cold maceration in ethanol 500 ml of 95% ethanol for 24 h in a (1L) ml conical flask with occasional shaking. The extract were filtered and concentrated in a fume chamber. The concentrated ethanol extract was used for the study.

Phytochemical analysis

A small quantity of the powdered lichen was subjected to phytochemical tests using established standard procedures for the determination of alkaloids, tannins, saponins, glycosides, flavornoids, carbohydrates, fats and oil using standard method (Harborne, 1998).

Preparation of the stock solution of the Lichen Extract and the colloidal silver

The stock solution of the lichen extract was prepared by weighting 40 mg (0.04 g) of the lichen extract and dissolving in 20 ml of DMSO to get a stock solution of 200 mg /ml. Two fold serial dilutions were made to give five different concentrations as follows: 200 to 6.25 mg/ml. for the colloidal silver, 1ml of the colloidal silver was diluted with 3 ml of distilled water to make a 4 ml stock solution of the colloidal silver in distilled water. According to the manufacturer instruction. The same serial dilution was made.

Preparation of standard culture of *staphylococcus aureus*

Clinical isolates of the microorganism were collected from the department of pharmaceutics, university of Nigeria, Nsukka. The organisms were then sub-cultured into manitol salt agar (MSA). To harvest the organisms, sterile normal saline was used. This was prepared by dissolving 0.9 g of sodium chloride in 100 ml of distilled water and the resulting solution sterilized by autoclaving at $121^{O}C$ for 15 min. 2 ml of sterile normal saline was added to the nutrient agar to obtain the microbial suspension. The microbial suspension thus harvested was taken to contain about 10^{9} colony forming units per 1 ml (cfu/ml) of the organisms. Subsequently appropriate serial dilutions of the resulting suspension were undertaken to get a microbial population of 10^{6} cfu/ml which was the standard inoculums size employed in this experiment.

Determination of the MICs of the lichen extract and colloidal silver solution

The sterile Petri dish was aseptically seeded with 0.1 ml of the standard culture (10^{6}cfuml) of *S. aureus* using a sterile standard pipette. 15 ml of sterile of molten agar was poured in the sterile plate and whirled to allow even distribution. The plate was divided into six different zone using marker and labeled A-F in a randomized manner representing the six serial dilutions of both solutions ranging from (200 to 6.25 mg/ml) and (4 to 0.125) of lichen extract and colloidal silver respectively. Two drops (0.02 ml per drop) of the different dilutions of the lichen extract and colloidal silver were placed in wells (8 mm diameter) bored on the nutrient agar plates already seeded using sterile cork borer. The plates were incubated at $37^{O}C$ for 24 h and then the inhibition zone diameters (IZDs) of the different concentrations of the solutions extract were measured and the MIC obtained by subtracting the diameter of the cup from the diameter of the zone of inhibition of each solution.

Determination of the combined effect of lichen extract and colloidal silver on *S. aureus* using agar plates method

The different concentrations of the extract with the stock solutions of colloidal silver were used in this experiment. An agar plate seeded with *S. aureus* was aseptically prepared. Two strips of absorbent filter paper were dipped respectively in to the solution of lichen extract and colloidal silver concentrate. The strips were removed from the solutions and allowed to drip-dry. The strips were then placed at right angles to each other and overlap on the agar, this was done for the rest of the serial dilutions of both colloidal silver and lichen extract. The plates were incubated at $37^{O}C$ for 24 h. The inhibition zone diameters (IZDs) were then measured and compared with the IZDs of the individual solution of colloidal silver and lichen extract alone

Data analysis

The data were subjected to the student's t-test at the p 0.05, level of significance by compared all the different ratios of the colloidal silver solution and the lichen extract.

RESULTS AND DISCUSSION

The result of the phytochemical analysis (Table1) shows that the lichen *P. perlata* contains flavonoids, saponins, tannins, glycosides (cardiac, cynogenetic but not the anthracene type), steroidal aglycone and carbohydrates but does not contain alkaloids.

The results from phytochemical analysis have been observed to be consistent with findings in several lichen species except that the anthracene glycosides which constitute an important class of lichen substances (Esimone et al., 1999) were absent; so also are the alkaloids. Other components found are abundant in several species of lichens (Esimone et al., 2007). Glycosides are found commonly in lichens and include the galactose-arabitol glycoside (umbilicin) and the galactose-mannitol (peltigeroside) both of which abound in different lichen genera.

The preliminary sensitivity test carried out showed that the ethanol extract *P. perlata* possesses antibacterial activity against the gram-positive bacteria, *S. aureus* as shown by the sensitivity and the MIC tests. The activities of the ethanolic extract against the test organism is concentration dependent (as shows in Table 2)

Table 2. MIC test with the lichen extract and admixture with colloidal silver solution.

S/N	Concentration Lichen extract (mg/ml)	IZD (mm) of Lichen extract (mg/ml)	Concentration of Colloidal silver solution (mg/ml	IZD (mm) of Colloidal silver solution (mg/ml	IZD (mm) of Mixture of Colloidal silver solution and lichen extract mg/ml
1	200.00	2.60± 0.01	4.0	1.50± 0.03	6.10± 0.01
2	100.00	2.20± 0.00	2.0	0.25± 0.02	4.60± 0.02
3	50.00	1.80± 0.02	1.0	0.05± 0.04	3.30± 0.00
4	25.00	1.60± 0.02	0.5	0.00± 0.00	2.50± 0.00
5	12.50	1.40± 0.00	0.25	0.00± 0.00	1.90± 0.05
6	6.25	0.80± 0.01	0.125	0.00± 0.00	1.10± 0.01

Mean IZD value for the test organism is significant at p 0.05, IZD = Inhibition zone diameter

Table 1. Phytochemical Tests on *Parmelia perlat*a.

Test	Inference
Alkaloids	-
Cyanogenetic Glycosides	+
Cardiac Glycosides	+
Anthracene Glycosides	-
Steroidal Glycosides	+
Saponins	+
Tannins	+
Flavonoids	+
Proteins	+
Carbohydrates	+
Reducing sugar	+

+ Presence, - Absence.

It can also be seen that colloidal silver at the concentration given as stock do have a minimal activity against the *S. aureus* based on the result of the preliminary and MIC tests. The combined effect of the agents show a remarkable effect on the test organism at concentration of 200 mg/ml of lichen extract and 4mg/ml of colloidal silver, the effect was synergistic, meaning that the combination of the two agents enhanced the activity of the lichen extract and these may lead to prevention of bacteria resistance organisms (Werner, 1992). This was observed as the IZDs of the lichen extract almost increased by two fold in the presence of the stock solution of the colloidal silver concentrate. Lichen has been show to possess many antimicrobial as well as antiviral properties (Huneck et al., 1996) . In related study, though against viruses (Kinchington et al., 1995), it has been reported that out of about fifteen indigenous Nigerian medicinal plants screened for antiviral activity by a vector-based assay technique, only extracts from the lichen *R. farinacea* exhibited potent antiviral activity against HIV-1 (an enveloped RNA virus) and adenovirus type 5 (a non-enveloped DNA virus). Researcher has shown that another tropical lichen from Nigeria also possesses antiviral activity, although against another viral family (Sydiskis et al., 1991). Research has shown that ointment containing extract of lichen *Ramalina farinaceae* exhibited antimicrobial activities against *Escherichia coli*, *Salmonnella typhi*, *Aspergillus niger* and *Candida albicans* (Ofokansi et al., 2005).

The rational involve in combined two antibiotics is to prevent monotherapy that often lead to bacteria resistance and in some case the combination broaden the bacterial activities against some other microorganism other than the staphylococcus especially in mixed infections. The advantage becomes very useful if the synergistic effect lead to bactericidal action of the agents.

The interactions above are when two or more antimicrobial agents are involved. However, the interaction between antimicrobial agents and non-microbial agent may enhance the killing or inhibiting effects of the antimicrobial agents on the microorganism. In some cases it may impair the overall antimicrobial activity (Klartersky, 1980).

It has been established, based on the finding from this ethanolic extract of the lichen *P. perlata* possess antimicrobial activities especially against S. *aureus* that is implicated in mixed infections and has shown high level of resistance to the commonly marketed antibiotics. It may be necessary for the future worker to design an *in vivo* situation in order to establish the usefulness of the extract in management of microbial infections.

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