PHYTOCHEMICAL PROPERTIES AND ANTIMICROBIAL ACTIVITIES OF *PHYSCIA GRISEA* ON CLINICAL ISOLATE OF *SALMONELLA TYPHI*

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ABSTRACT
Physcia grisea were ground, extracted with ethanol, and some bioactive components determined before the extract was screened for phytochemical and antimicrobial activities on *Salmonella typhi* using Agar Cup Diffusion Technique. Chloramphenicol was used as a standard antibacterial agent for comparison. The phytochemical compounds found in the extract include tannins, flavonoids and alkaloids. The result of antimicrobial screening showed that *P. grisea* has antibacterial activity against *S. typhi* like chloramphenicol. However, *P. grisea* has a lower antimicrobial action than chloramphenicol.
Keywords: *Physcia grisea,* phytochemical, antimicrobial activities and *Salmonella typhi*

INTRODUCITON
Medicinal plants have provided opportunities for new drugs because of their matchedless availability of chemical diversity (Abad *et al*, 2007). Quinine, Penicillin, artesenate and many others are good examples of these plant derived drugs. Thus, considerable information now exists on the phytochemical constituents and antimicrobial activities of most well known medicinal plants. There is however little or no information regarding the phytochemical constituents of *P. grisea* despite the facts that its medicinal uses in HIV/AIDS cases have been extensively reviewed by Eze *et al* (2009). Besides, knowledge of phytochemical substances naturally present in plants that are or may be used as drugs are useful in evaluating pharmacodynamics profiling. *P. grisea* is lichen found on walls, rocks, and trees, attached by short threads which grow from the underside and are white with black tips.

The plant is light grey or slightly brownish grey, and is almost always covered, at least near the tips of the lobes, with a very fine white powder. The colour develops a greenish tinge when the plant is wetted (Nicholson, 1966). Since extensive use of antibiotics has favoured the emergence of resistant strains of *S. typhi*, there is a need for a novel antimicrobial agent that will be cheap, stable and better in the treatment of salmonelloisis caused by this pathogen and hence this work. Besides, this study compared the antibacterical activities of *P. grisea* extract with chloramphenicol which is a standard antibiotics.

MATERIALS AND METHODS
Test Organism: the microorganism used for this research was *S. typhi* obtained from microbiology laboratory in the Department of Vetinary Medicine, University of Nigeria, Nsukka.

Reagents
The following reagents were used; chloramphenicol, *P. grisea* extract, ethanol. The culture media used were nutrient agar and nutrient broth (Oxoid).

Sources of samples
The lichen, *P. grisea* used for this research was obtained from the back of *Dialum guinense* tree in Amogu-Ezimo Uno in Udenu LGA, Enugu State, Nigeria and identified in the department of Botany, University of Nigeria, Nsukka.

Method of Extraction
Thirty-seven grams of the pulverized *P. grisea* were weighed out using mettler sensitive balance and poured into 750 ml flat bottom flask and these were soaked in 500 ml of absolute ethanol. This was stirred with magnetic stirrer for 18 hours and left to stand for 24 hours before it was filtered using a clean muslin cloth and then concentrated in the oven (Gallenkamp, England) at 60 ^{0}C.

Preliminary Sensitivity Test
The preliminary sensitivity test of the *P. grisea* extract and chloramphenicol, were evaluated by the bore plate and agar diffusion method as described by Agboke *et al* (2005).

Determination of IZD of *P. grisea,* on *S. typhi* Using Agar Cup Diffusion Technique
Sterile Petri dishes were aseptically seeded with 0.1ml of freshly prepared suspension of *S. typhi* using 20 ml of nutrient agar and swirled three times in clockwise and anticlockwise direction to ensure an even distribution of the test organism and was allowed to set. One gram of *P. grisea* extract was dissolved in 2.5 ml of DMSO and a four 2 fold serial dilutions were made. These dilutions were labelled 1, 2, 3, 4,. After this, four different solutions with different concentrations were prepared for sensitivity testing.

The agar plate was divided into four sections using a marker and labelled 1, 2, 3, and 4 to represent the different concentrations got from the serial dilutions. With a Cork borer, cups were made at the centre of each of the four sections marked, and then 0.05 ml each of the dilution of *P. grisea* was added into the cups using a sterile dropper starting from the lowest to the highest concentration. The plate was labeled and incubated at 37 ^{0}C for 24 hrs, the zones of inhibition (IZD) were measured using a metre rule and were recorded. The graph of 1ZD square against the logarithm of concentration was plotted and the MIC calculated.

Determination of 1ZD of Standard Antimicrobial Agent on *S. typhi*
The preparation of agar, seeding of plate and making of agar cups were done using the same method described above. The standard antimicrobial agent used was 500mg chloramphenicol dissolved in 25ml of distilled water to get the initial concentration of 20mg/ml. the drug solution was serially diluted into 3 two folds and IZD determined as in the above.

Determination of Phytochemical Compounds
Phytochemcial test were carried out to determine the bioactive constituents present in the plant extract.

Determination of Alkaloids

Alkaliods were determined using the method described by Harbone (1973). One gram of *P. grisea* extract was weighed into 25 ml beaker then 20 ml of 10 % acetic acid in ethanol was introduced into it and covered to stand for 4 hrs after which it was filtered. Thereafter, 10 ml of ammonium hydroxide was introduced into the filtrate to precipitate the alkaloids. The precipitate was filtered, weighed and the percentage alkaloid calculated

Determination of Flavonoids

Flavonoids were determined using the method described by Boham and Kocipai, (1974). One gram of *P. grisea* was weighed into a flask and the flavonoid was extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature. Thereafter, it was filtered with whatman filter paper No 42 (125mm) and the filtrate transferred into a weighed beaker and evaporated to dryness to get the weight of the flavonoids. The percentage flavonoid was then calculated.

Determination of Tannins

Tannins were determined using the methods described by Pearson, (1976). One gram of *P. grisea* was measured into a flask with 10 ml of distilled water introduced into the sample; it was allowed to stand for 30 min at room temperature with gentle shaking at every 5 min intervals. The solution was centrifuged after which 2.5 ml of the supernatant was measured into a 50 ml volumetric flask, similarly 2.5 ml of standard tannin solution was measured into a separate 50 ml flask, and then 1.0 ml of Folin-Denis reagent was added into each flask followed by 2.5 ml of saturated sodium bicarbonate solution. The mixture was incubated for 90 min at room temperature. Thereafter, the absorbance reading was read and the percentage tannin calculated.

Statistical Analysis

Student's t – test was used for comparison between the two treatments. A difference was considered statistically significant when $P < 0.05$.

RESULTS

The result of the preliminary sensitivity tests of *P. grisea* extract and chloramphenicol are shown below. The preliminary test showed that *S. typhi* was moderately sensitive to *P. grisea* extract and highly sensitive to chloramphenicol.

Sensitivity of *S. typhi* to the antimicrobial agents

Antimicrobial agent	*S. typhi*
P. grisea extract	++
Chloramphenicol	+++

++ *S. typhi* was moderately sensitive to *P. grisea* extract

+++ *S. typhi* was highly sensitive to chloramphenicol.

Effects of different concentrations of *P. grisa* extract on *S. typhi*

Conc(mg/ml)	1ZD(mm)	IZD^2 (mm^2)	Log Conc (mg/ ml)
400	15.00	225	2.6021
200	13.00	169	2.3010
100	10.00	100	2.00
50	9.00	81	1.6989

Values are mean of three replicates from three trials after 24 hours of incubation.

Effects of different concentrations of chloramphnicol on *S. typhi*

Conc(mg/ml)	IZD(mm)	IZD^2 (mm^2)	Log Conc (mg /ml)
S20	30.00	900	1.301
10	24.00	576	1.00
5.0	22.00	484	-0.6989
2.5	20.00	400	-0.3979

Values are mean of three replicates from three trials after 24 hours of incubation.

Percentage composition of Phytochmicals in *P. grisea*

Phytochmical	**Percentage composition**
Alkaloid	24.0
Flavonoid	70.0
Tannin	6.0

MIC of *P. grisea* extract

The minimum inhibitory concentration (MIC) of the *P. grisea* extract was calculated from the graph of IZD^2 against log concentration of the extract to be 19.45mg/ml.

MIC of Chloramphenicol

The minimum inhibitory concentration (MIC) of the standard antimicrobial agent, chloramphenicol, was calculated from the graph of the IZD^2 against log concentration of chloramphenicol to be 5.37mg/ml.

DISCUSSION

The results of the phytochemical analysis of the extract showed that *P. grisea* has flavonoids, alkaloids and tannins with flavonoids being the most abundant. These are the most important bioactive constituents of plants (Hill, 1952). The high flavonoid contents of the extract mean that it may have high antioxidant activity; since flavonoids are modifiers which modify the body's reactions to allergens, viruses, and carcinogens (Malu *et al*, 2009).

The tannin content of the extract was 6.0 percent. Although tannins may decrease protein quality by decreasing digestibility, and palatability, they have good antimicrobial activities. Besides, complex flavonoid polymers which are condensed tannins were high in *P. grisea* extract and they (flavoniods) have antiallergic, anti-inflammatory, antimicrobial and anticancer activity (Balch and Balch 2000) and may be useful in therapeutic roles (Jisika *et*

al, 1992.). Thus, *P. grisea* could represent a led source of drugs for viral, bacterial and fungal treatments.

Analysis carried out on *P. grisea* in this research also showed that it has 24 percent alkaloids which are basic natural products occurring primarily in plants. Alkaloids have sedative and analgesic properties (Malu *et al* 2009). Since *P. grisea* contains alkaloids which possess important physiological properties, it may be used in producing pain relieving drugs.

The results of the antimicrobial activity of the extract showed that it had the highest inhibition zone diameter (IZD) at 400 mg/ml which was the highest concentration used. This is in line with the observation of Eze *et al* (2009) that the antimicrobial activity of *P. grisea* is concentration dependent.

From this study, it was observed that *P. grisea* showed antimicrobial activity on *S. typhi,* though comparatively lesser in action than the standard antimicrobial agent (chloramphenicol) used. This means that *P. grisea* extract could give a therapeutic effect in the treatment of infections caused by *S. typhi* if properly utilized. Although the differences in their actions were statistically significant ($P<0.05$), it may have resulted from the crude nature of *P. grisea* extract.

It is therefore recommended that *P. grisea* extract has good antibacterial potentials and should be fully exploited for its suitable applications on clinical trials.

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