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Evaluation of phytochemical constituents and hepatoprotective effects of *Physcia grisea* in albino rats

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Abstract

Objectives: The study aimed to evaluate the phytochemical constituents and hepatoprotective effects of *Physcia grisea* in albino rats. **Methods/statistical analysis:** The analysis of phytochemical constituents of *P. grisea* was carried out to determine the level of tannins, phenols, terpenes, flavonoids and alkaloids present in the sample. The blood samples from four groups (A, B, C, and D) of albino rats with each group containing four per group were analyzed for Aspartate aminotransferase (AST) and bilirubin secretions which are two early indicators of liver injury. The analysis was carried out in two phases; phase 1 and phase 2. In phase I, which was regarded as the base line analysis, the weights of the animals were taken and the blood samples were collected following ocular puncture. In the second phase of the analysis, the group A, B and C were administered 500 mg/kg, 250 mg/kg and 125 mg/kg concentrations of *P. grisea* respectively while the control group was administered only tragacanth solution through the oral route. After 3 weeks of the administration, their blood samples were collected for another analysis using the same method of blood collection. The data obtained were analyzed using GenStat Release 10.3DE (2011) statistical software. **Findings:** The results showed varying levels of phytochemicals in *P. grisea*, alkaloids (36.6%), flavonoids (3.6 %), saponins (2.7 %), total phenols (25.15 %), tannins (0.029 %) and terpenes (2.0 %). The results of the *in-vivo* studies showed that *P. grisea* had no significant effect on the AST and bilirubin secretions at 125mg/kg after 3 weeks of the administration. This was an indication that there was no liver injury at this dose within the period of administration. **Application/improvements:** If properly harnessed, *P. grisea* extract could provide a novel and cheap source of medicine for treatment of liver diseases resulting from AST and bilirubin secretions.

Keywords: Phytochemical; *Physcia grisea*; Aspartate Aminotransferase; Bilirubin

1 Introduction

The use of plant products in conventional human therapies dates back at least to the Neolithic age. For this reason, there are well-documented plant products used in the treatment of various infections and disorders of man and animals in many parts of the world⁽¹⁾. Quinine and penicillin drugs are good examples of medicinal products from plants that have been used successfully in the treatment of human infections.

In recent years, increasing attention has been given to medicinal products from plants due to their cheap and diverse bioactive compounds that are of pharmaceutical value. In Nigeria, many people in both urban and rural areas are familiar with one plant medicinal product or the other as they selectively exploit them for treatment of human infections and disorders. Thus, virtually, all plant phyla from thallophytes to the higher phyla are being investigated to ascertain their medicinal attributes⁽²⁾. The plant *Physcia grisea* is a thallophyte of the order Lecanorales and in the family Physciaceae. It is commonly called Lichen and it has abundant medicinal properties⁽³⁾. The diversity of medicinal uses of *P. grisea* has received early attention in studies carried out on HIV/AIDS patients by⁽⁴⁾. There are also many phytochemical compounds with therapeutic properties that have been identified to be present in *P. grisea*⁽¹⁾. Available information on *P. grisea* showed that it has good antifungal and antibacterial properties as well as many antioxidant compounds which can be protective to body organ such as the liver⁽⁴⁾.

P. grisea is a lichen found on walls, rocks and trees, attached by short threads which grow from the underside and are white with black tips. *P. grisea* 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⁽⁵⁾. *P. grisea* possesses a broad spectrum of medicinal properties and could represent a novel source of drugs belonging to a wide range of structural classes that can be used in the treatment of many diseases⁽³⁾. Despite the enormous use of *P. grisea*, there is still little or no information on the effects of the plant on liver functions, hence this work. Although many users of plant medicinal products tend to believe that they are inherently safe, a good number of the products can cause kidney and liver problems⁽⁶⁾. Besides, *P. grisea* possesses many phytochemical compounds such as tannins, phenols, terpenes, flavonoids and alkaloids which are capable of inducing physiological changes in body cells⁽¹⁾. The findings of⁽⁷⁾ on the beneficial effects of *P. grisea* on renal function also called for the need to investigate the hepatoprotective effects of the plant and hence this study.

Bilirubin formerly referred to as hematoidine is the yellow breakdown products of normal heme catabolism. It is a lipid-soluble substance usually carried in plasma as a plasma protein-bound substance⁽⁸⁾. In certain condition, the concentration of pigment increases and causes jaundice (icterus) when the skin, sclera of the eye and body fluids become pigmented yellow⁽⁹⁾. It is produced when the liver breakdown old red blood cells. Bilirubin is removed from the body through the faeces (stool) and gives stool the normal brown colour. Aspartate aminotransferase (AST) also called serum glutamic oxaloacetic transaminase (SGOT) is an enzyme found in a diversity of tissues such as the heart, liver, skeletal muscle, pancreas, kidney and red blood cells⁽¹⁰⁾. It is an aminotransferase because; it transfers an amino group into ketoacids. It is found predominantly in the cytoplasm and mitochondria. They are released into the blood when the organs or tissues where it is found are injured. The amount of AST is directly related to the number of cells affected by, tested after the injury. In diagnosis, the presence of elevated aspartate aminotransferase can be an indicator of liver and cardiac damage⁽¹¹⁾. Thus, substances such as drugs or plant extracts that can cause secretion of high level of aspartate aminotransferase in the liver, may cause liver damage while those that help the liver to produce optimum secretion of aspartate aminotransferase could be investigated to play a hepatoprotective role and hence, this work.

2 Materials and methods

The reagents used for this research were all analytical grades from Sigma-Aldrich (Germany) and those that were commercially prepared by Randox Laboratories Ltd, United Kingdom. The equipment used was hot air oven (Baid and Tatlock, England, Model no: BS 2648), UV-Visible spectrophotometer (Cole-Parmer, PEC Medical USA, Model no: UV-7504), water bath (GCA/Precision Scientific, Chicago, USA. Model no: 66800) and electronic weighing balance (HX China, Model no: HX103T).

Experimental animals

The animals used were male and female adult albino rats. The animals were bought from the Department of Zoology, Faculty of the biological Sciences, University of Nigeria, Nsukka. The animals were acclimatized for two weeks at room temp (28°C).

Collection and preparation of *P. grisea* leaf

The *P. grisea* used for the study was obtained from Ezimo-Uno Community, Udenu Local Government Area of Enugu State and was identified in the department of Crop Science, University of Nigeria, Nsukka. The voucher specimen of the lichen understudy

was deposited in the herbarium of the Department of Crop Science, University of Nigeria, Nsukka. The leaves were air dried for about four weeks after which they were pulverized before they were extracted with 96 % ethanol.

Method of extraction

The method of⁽¹⁾ was used in the extraction of the pulverized *P. grisea* sample. Thirty-seven grams of the pulverized *P. grisea* were weighed out using an electronic weighing balance (HX China, Model no: HX103T) and poured into a 750 ml flat bottom flask. Thereafter, 500 ml of absolute ethanol was added into the 750 ml flat bottom flask and stirred with a magnetic stirrer for 18 hours. It was then allowed to stand for 24 hours before it was filtered using a clean muslin cloth and then concentrated in the oven (Baid and Tatlock, England, Model no: BS 2648) at 60[°]C.

Determination of the phytochemical constituents of the *P. grisea*

The alkaloid, flavonoid, tannin, saponin and total phenol contents of *P. grisea* were determined quantitatively as described below.

Determination of Alkaloid

The alkaloid contents of the *P. grisea* were determined as described by⁽¹²⁾. One gram of air dried pulverized/powdered *P. grisea* sample was weighed into 100 ml beaker and 10 ml of 10 % acetic acid in ethanol added into it and covered. It was then allowed to stand for 4 hours after which it was filtered. Thereafter, 5 ml of ammonium hydroxide (NH₄OH) was introduced into the filtrate to precipitate the alkaloids. The precipitate was filtered, weighed, dried and the percentage alkaloid calculated.

$$\text{Alkaloid (\%)} = \frac{(\text{weight of the filter paper} + \text{Alkaloid}) - (\text{weight of filter paper only})}{\text{Weight of ground sample used}} \times \frac{100}{1}$$

Determination of Flavonoids

The flavonoid contents of the *P. grisea* were determined as described by⁽¹³⁾. One gram of the powdered *P. grisea* sample was weighed into a flask and the flavonoids in the test samples were extracted repeatedly with 20 ml of 80 % aqueous methanol at room temperature. Thereafter, it was filtered using whatman filter paper No 43 (125 mm) and the filtrate was evaporated in weighed beaker to dryness to get the weight of the flavonoids in the test samples. The percentage flavonoids were calculated.

$$\text{Flavonoid (\%)} = \frac{(\text{weight of beaker} + \text{flavonoid}) - (\text{weight of beaker only})}{\text{Weight of sample used}} \times \frac{100}{1}$$

Determination of Tannins

The tannin present in the *P. grisea* was determined as outlined by⁽¹⁴⁾. One g of the powdered *P. grisea* sample was weighed and 10.0ml of distilled water was added. This was shaken at 5 min intervals for 30 min. The solution of the sample was centrifuged or filtered to get the extract. Then, 2.5 ml of the supernatant was transferred into a test-tube. This was added 1.0 ml Folin-Denis reagent, followed by 2.5 ml of saturated Na₂CO₃ solution. Thereafter, 2.5 ml of standard tannic acid solution and reagent blank were prepared and the absorbance read at 250 nm wavelength after 90 min of incubation at room temperature. The percentage (%) tannin was calculated thus:

$$\% \text{ Tannin} = \frac{A_n}{A_s} \times C \times \frac{100}{W} \times \frac{v_f}{v_a}$$

Where A_n = absorbance of test sample, A_s = absorbance of standard solution, C = Concentration of standard solution, W = weight of sample used, V_f = total volume of extract, V_a = volume of extract analyzed in millilitre (ml).

Determination of Saponin

The method of⁽¹⁵⁾ was used for the determination of the saponin in the samples. Two g of powdered *P. grisea* sample was weighed into a conical flask. This was added 100 ml of 20 % aqueous ethanol. The solution of the sample was heated in a hot water bath (GCA/Precision Scientific, Chicago, USA. Model no: 66800) for 4 hr with continuous stirring at about 55 °C. This was filtered and then re-extracted with another 200 ml of 20 % ethanol. The extract was concentrated in the water bath to 40 ml at 90 °C before 20 ml of diethyl ether was added and shaken vigorously to separate the aqueous layer. The aqueous layer was

recovered and 60 ml of n-butanol added and then washed twice with 10 ml 5 % aqueous sodium chloride. This was evaporated in the oven and then quantified in percentage.

Determination of total Phenol

The total phenol present in the test sample was determined quantitatively as described by⁽¹⁵⁾. Two g of powdered *P. grisea* sample was weighed into a flask, de-fated with petroleum ether and allowed to stand for few minutes to air dry. The air-dried de-fated samples were boiled with 50ml of diethyl ether for 15min. to extract the phenol and 5 ml of the aliquots of the boiled sample transferred into test tube. This was added 2 ml of NH₄OH solution, 5ml of 99 % amyl alcohol (C₂H₅C(CH₃)₂OH) and allowed to stand for 30 min. for colour development before the sample's absorbance was read at 505 nm wavelength using UV-Visible spectrophotometer (Cole-Parmer, PEC Medical USA, Model no: UV-7504). The standard and the reagent blank were prepared by adding all the reagents put in the test samples except that the test sample was replaced with a standard phenol solution and distilled water, respectively. The concentration of phenol in the test sample was then calculated using the formula below. Calculation:

$$\text{Phenol (mg/100 g)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{1}$$

Administration of *P. grisea* leaf extract to the animals

The animals were divided into four groups (A, B, C and D). Each group contains four albino rats with group D serving as the control. Their respective weights were taken before the administration of the leaf extract. The rats were administered the dried leaf extract of *P. grisea* using oral gavage morning and evening daily for three weeks. Group A, B, and C were administered 500 mg/kg, 250 mg/kg and 125 mg/kg of the leaf extracts, respectively using 10 % tragacant as the vehicle and group D (control) was administered 10 % tragacant solution only. Thereafter, their respective weights were taken again after three weeks of the administration of the extract.

Determination of Aspartate Aminotransferase (AST) and Bilirubin

Aspartate aminotransferase (AST) and bilirubin levels were determined in the animal's blood before and after administration of the extract, using kits from Randox Laboratories Ltd, United Kingdom.

Statistical analysis

The data obtained were analyzed statistically with⁽¹⁶⁾ statistical software.

3 Results and Discussion

The phytochemical contents of the *P. grisea* is represented in Table 1. The percentage alkaloids present in the *P. grisea* is higher (36.6 %) than other medicinal plants such as *Gongronema latifolia* and *Moringa olifera* with 9.0 % and 3.07 % alkaloids, respectively. Alkaloid is a plant natural product that produces sedative and analgesic effects and has been useful in the production of medicines. Plant alkaloids have therefore been harnessed as a useful raw material for the production of pain-relieving drugs⁽¹⁾. The percentage flavonoid contents of the *P. grisea* were which was high in rich antioxidants⁽¹⁷⁾ and could play a beneficial role in the reduction of oxidative stress⁽¹⁸⁾. Oxidative stress is considered to play a pivotal role in the pathogenesis of aging and several degenerative diseases, such as atherosclerosis, cardiovascular disease, type II diabetes and cancer⁽¹⁹⁾. Flavonoids have also been reported to have protective effects against cancer cells⁽²⁰⁾. This is because flavonoid facilitates the excretion of cancer conjugating enzymes in metabolic waste products. The tannin content of the *P. grisea* was low. Tannins which are plant polyphenolic substance with molecular weight greater than about 500 inhibit protein absorption in the gastric system⁽²¹⁾. The low value of tannins will have little or no influence on bio availability of *P. grisea* if processed to be taken as a drug. Little quantity of tannins from plant extracts has been reported to play therapeutic roles in both human and animal trials⁽¹⁹⁾. The high level of the phytochemicals such as alkaloid, flavonoid and saponnin in the *P. grisea* might therefore be responsible for the high medicinal values of the plant.

Table 1. Concentration of phytochemical constituents of *P. grisea* in Percentage

Phytochemicals	%
Alkaloids	36.6
Flavonoids	3.60
Tannins	0.029
Saponins	2.70
Total phenols	25.15
Terpenes	2.00

Values are mean of three replicates analysis

The results of the analysis carried out on the blood samples from the albino rats for the determination of Aspartate aminotransferase (AST) and Bilirubin secretions before and after the experiment are shown in Tables 2 and 3 below.

Table 2. Concentration of AST and Bilirubin in the Blood of the Albino Rats and their Weights at Baseline Analysis

Groups	Rats	Conc.of ast(u/l)	Conc. Of bil(mg/dl)	Weights(g)
A	A1	12.60	0.994	200
	A2	12.60	1.080	200
	A3	12.60	0.346	150
	A4	12.60	0.994	200
B	B1	12.60	3.564	150
	B2	12.60	1.564	250
	B3	12.60	4.104	150
	B4	12.60	4.536	150
C	C1	12.60	0.864	300
	C2	12.60	1.404	150
	C3	12.60	6.264	200
	C4	12.60	3.024	150
D	D1	12.60	0.200	150
	D2	12.60	0.210	150
	D3	12.60	0.250	200
	D4	12.60	0.230	150
LSD (p<0.05)		n.s	2.145	72.1

Values are replicates from four trials

Table 3. Concentration of AST and Bilirubin in the Blood of the Albino Rats and their Weights after Administering the Extract for 3 Weeks

Groups	Rats	Conc.of ast(u/l)	Conc. Of bil(mg/dl)	Weights(g)
A	A1	12.60	15.034	250
	A2	12.60	15.120	250
	A3	12.60	14.386	250
	A4	12.60	15.034	250
B	B1	12.60	8.748	200
	B2	12.60	9.180	275
	B3	12.60	9.288	200
	B4	12.60	9.720	200
C	C1	12.60	2.484	300
	C2	12.60	1.620	175
	C3	12.60	6.480	200
	C4	12.60	3.240	200
D	D1	12.60	0.210	200
	D2	12.60	0.230	200
	D3	12.60	0.256	250
	D4	12.60	0.221	200
LSD (p<0.05)		n.s	1.684	55.03

Values are replicates from four trials

The results of the analysis of the blood samples collected from the rats after they have been orally administered *P. grisea* leaf extract for three weeks showed that there was no significant difference ($p > 0.05$) in AST secretions when compared with the baseline analysis. According to⁽¹⁰⁾, little or no secretion of AST indicates the absence of injury in the liver cells. This showed that there was no injury to the liver cells throughout the three weeks of administration of *P. grisea* extract. There was an increase in the total bilirubin secretions after three weeks of administration of *P. grisea* extract to the rats. According to⁽²²⁾, in the absence of liver injury or disease, high levels of total bilirubin confer various health benefits. Several research findings have also shown that levels of serum bilirubin are inversely related to the risk of certain heart diseases^(23,24). This means that the extract may have healing effects on the liver.⁽²⁵⁾ reported that extracts with healing properties on the liver will always have no significant difference in the AST and bilirubin secretions after three weeks of administration. There was an increase in the weights of the rats after the administration of *P. grisea* extracts. This may have resulted from the growth and development in the rats' cells due to advancement in their age. In⁽²⁶⁾ reported that maturity of the rats, as well as, the presence of some nutrients in some plant extract can increase rat weights. The increase in the weights of the rats could also be as a result of the ability of *P. grisea* extract to increase red blood cell production⁽²⁵⁾. Thus, *P. grisea* could represent a led source of a natural medicinal product if properly utilized⁽²⁷⁾. Besides, *P. grisea* extract could contain some phytochemicals that facilitate erythropoietic process in rats. This can be seen from the significant increase in the weights within 21 days of the administration of the extract. These qualities of *P. grisea* have been suggested to be safe and could be used in drug production⁽²⁸⁾. Overall, the effects of 125 mg/kg of the extract gave the best result on liver function.

It is therefore concluded that *P. grisea* has numerous health benefits, such as protecting liver cell injuries and certain heart diseases, that can be harnessed in both orthodox medicine and pharmaceutical sector but the users should be mindful of the safe dosage in both animal and human therapeutics for proper organ function. The protective activity of the *P. grisea* on the liver could be attributed to antioxidant compounds present in the plant⁽⁴⁾. Many polyphenolic compounds such as flavonoids play antioxidant roles by neutralizing oxidative activities in the body by donating an electron or hydrogen atom to the free radicals⁽¹⁹⁾. This can reduce injury in body organs such as liver or heart as observed in this study and even facilitate the excretion of cancer conjugating enzymes in metabolic waste products.

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