Al-Qadisiyah Journal of Pure Science

Manuscript 1240

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ARTICLE

Preliminary Phytochemistry, *In-vitro* Antioxidant and Reducing Effect of Intraocular Pressure of *Pleurotus tuber-regium* (Fr) Sing Using Animal Model

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Abstract

This study investigate the qualitative and quantitative phytochemicals, antioxidant screening, and the reducing effect of intraocular pressure of *P. tuber-regium* aqueous extract in rabbits. Standard protocols were used to analyze the qualitative and quantitative phytochemical, *in-vitro* antioxidant studies ferric reducing antioxidant power (FRAP), total antioxidant capacity) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were also used to assess these plants. Thirty (30) New Zealand male rabbits were randomly selected for this study. The whole group except normal control were induced with 1% Prednisolone acetate suspension for 3 days to obtain the baseline intraocular pressure (IOP) >5−10 mm Hg, afterward treated with a graded oral dose of 100, 200, and 400 mg/kg *P. tuber-regium* respectively. The application was administered daily in the right eye (OD) only for 7 days, and the IOP measurements were. The phytochemical screening of the extract showed the presence of flavonoids, phenol, tannins, and saponins. Tannins and phenol were abundant (28 and 20 mg/kg). The antioxidant scavenging property showed the potential capacity of *P. tuber-regium* extract to scavenge free radicals against oxidative stress at 10%, 100%, and 25.26% of *P. tuber-regium* aqueous extract when compared with ascorbic acid. The result from this present study at graded doses (100, 200, and 400 mg/kg) of *P. tuber-regium* aqueous extract elicited a significant decrease in intraocular pressure levels, specifically at 200 and 400 mg/kg of the mushroom extract had a markable reduction in the mean IOP level when compared with timolol control and untreated control ($p < 0.05$). Also, *P. tuber-regium* aqueous extract exhibited a prolonged duration of action with an increased half-life and sustained therapeutic effect as shown in days 10−28. In conclusion, *Pleurotus tuber-regium* aqueous extract elicited intraocular pressure-lowering effect in rabbits, which validate its ethnomedicinal claim on ocular hypertension.

Keywords: Phytochemistry, *In-vitro* antioxidant, Intraocular pressure, *Pleurotus tuber-regium*

1. Introduction

Herbal medicine is the oldest form of health care system since antiquity [1,2]. One-third of nations' adults use herbal medicine. This explains why most medicines were produced directly from natural sources such as plants, water, earth, sun, and animals. Medicinal plants have been observed to be most widely used in many developing countries for the management of several disease condition, and maintaining good health [3]. Furthermore, pharmaceutical industries now depend largely on medicinal plants as a raw materials for the production of drugs. This has been traceable to the extraction and development of several therapeutic drugs from plants materials [4]. Studies have shown that factors responsible for non-compliance to treatment regimens include: the high cost of conventional medicine, lack of availability, and their adverse effect. Hence, the need

Received 24 September 2023; accepted 11 April 2024.
Available online 28 September 2024

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https://doi.org/10.29350/2411-3514.1240

for an alternative regimen that is affordable, available with less or no adverse effects [5–7] (Figs. 1–7).

Intraocular pressure (IOP) is the magnitude of the force exerted by an ocular fluid (aqueous humor) on the internal surface area of the anterior segment of the eye [8]. Its normal value ranges between 12 and 21 mmHg with a mean value of 16 mm/Hg. It is regulated by the dynamics between aqueous secretion from the ciliary body and its outflow through the trabecular meshwork. The process involves the physiological pumping of ions and molecular substances (i.e., organic and inorganic) of small and large sizes across the epithelial cell membrane covering the ciliary process. An imbalance in the dynamics of aqueous production could cause a change in intraocular pressure [9–14]. It is worthy of note that a positive correlation exists between systemic pressure and IOP because of the variation in hydrostatic pressure within the ciliary capillaries. Hence, a sudden change in systemic blood pressure would likely break through to the capillaries and cause a similar change in the intraocular pressure [15–17].

Pleurotus tuber-regium (Fr) Sing belongs to the family "Pleurotaceae", genus Pleurotus and it means "side ear" because of its attachment cap to the substrate. It is known as the "tiger milk mushroom," "sclerotia-producing *Pleurotus*," a nematode-trapping mushroom, and it is also

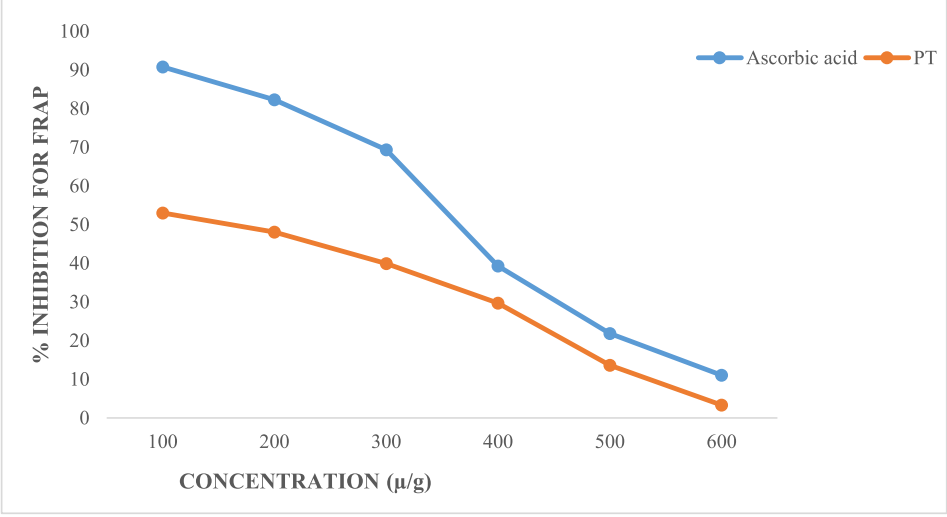

Fig. 1. Effect of Pleurotus tuber-regium *extract on ferric reducing antioxidant power.*

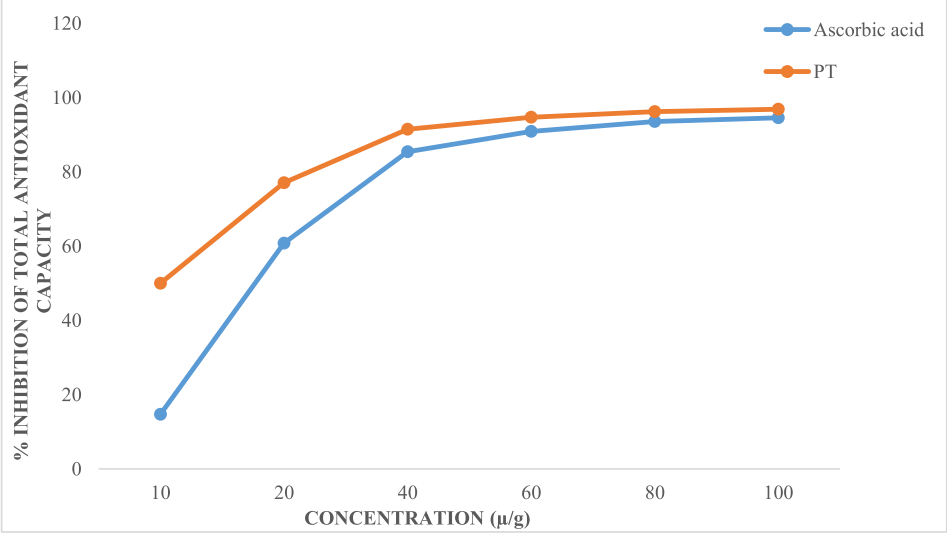

Fig. 2. Effect of Pleurotus tuber-regium *extract on total antioxidant capacity.*

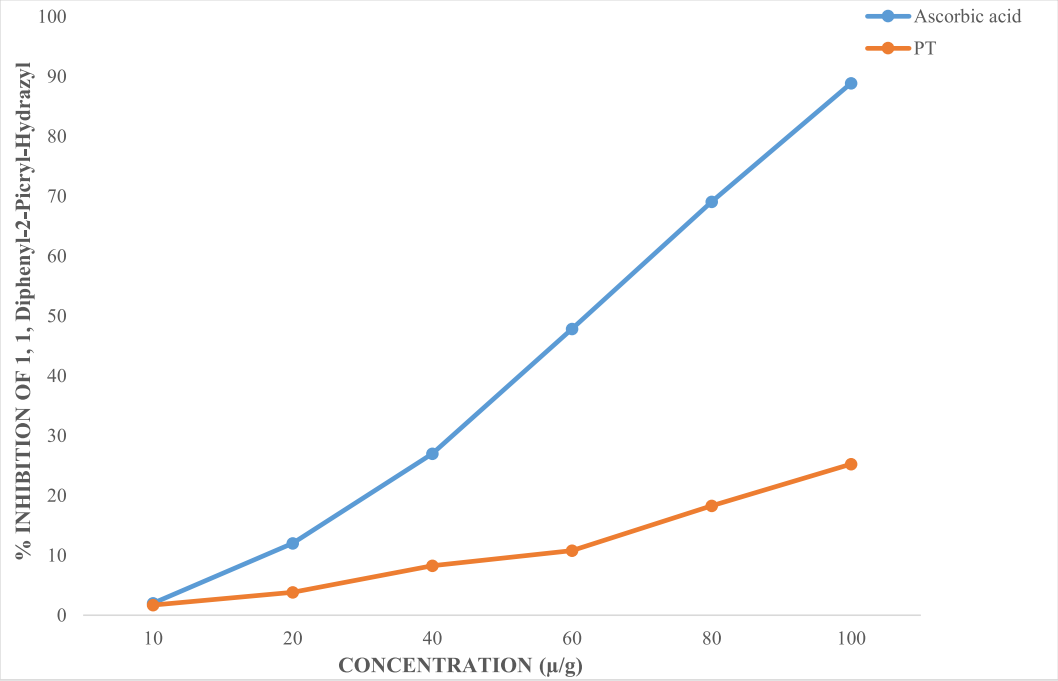

Fig. 3. Effect of Pleurotus tuber-regium *extracts on1, 1, Diphenyl-2-Picryl-Hydrazyl antioxidant.*

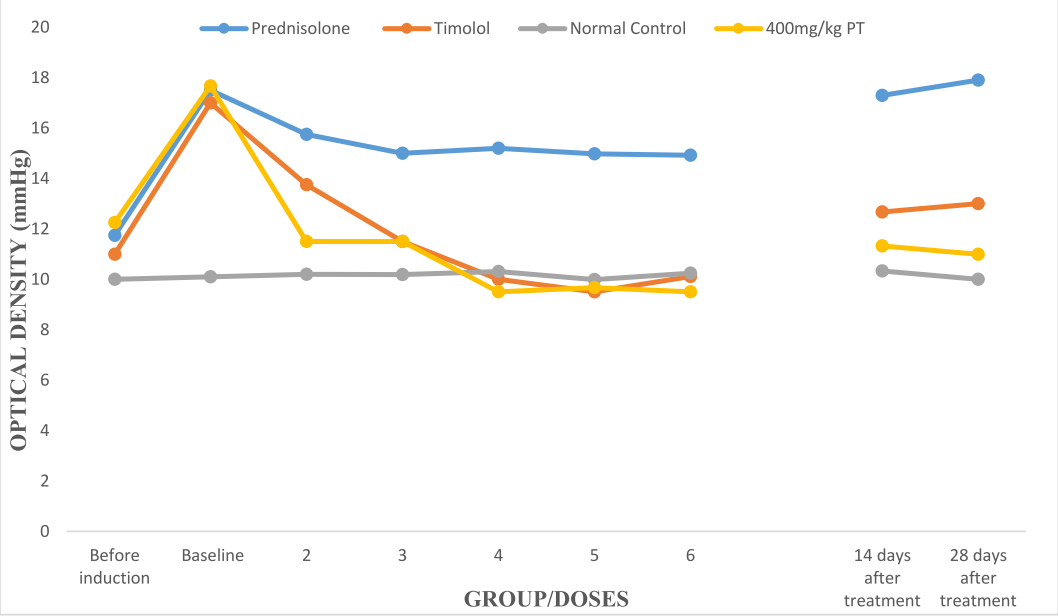

Fig. 4. Effect of Pleurotus tuber-regium *in ocular pressure at highest doses of treatment.*

identified as the "King Tuber Oyster mushroom" in China [18]. In Nigeria, it is called "osu" or "ero nsu" in Igbo, "ohu" in Yoruba, and "katala" or "rumbagada" in Hausa languages. *P. tuber-regium* is native to the tropical and subtropical regions of Africa, Asia, and Australia-Pacific [19–21]. Phytochemical analysis of *Pleurotus tuber-regium* shows that it contains alkaloids, saponins, flavonoids, tannins, anthraquinones, and phytate. D-Mannitol is also a phytochemical property contained in *P. tuber-regium* to inhibit the activity of angiotensin1 converting enzyme, making it possess an antihypertensive effect. Other active phytoconstituents include; peptides, polysaccharides, glycoproteins,

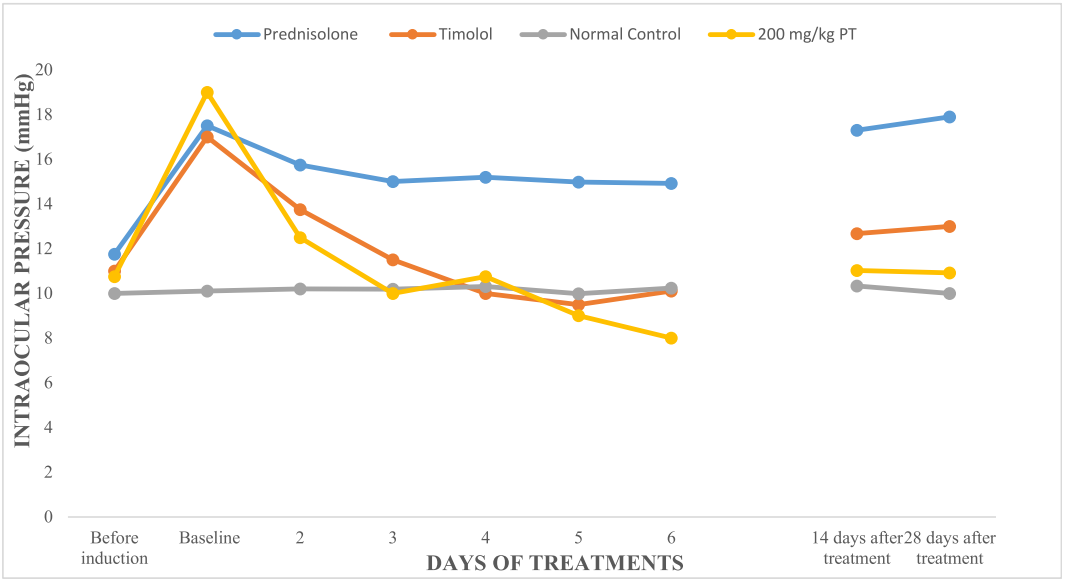

Fig. 5. Effect of Pleurotus tuber-regium *in ocular pressure at media doses of treatment.*

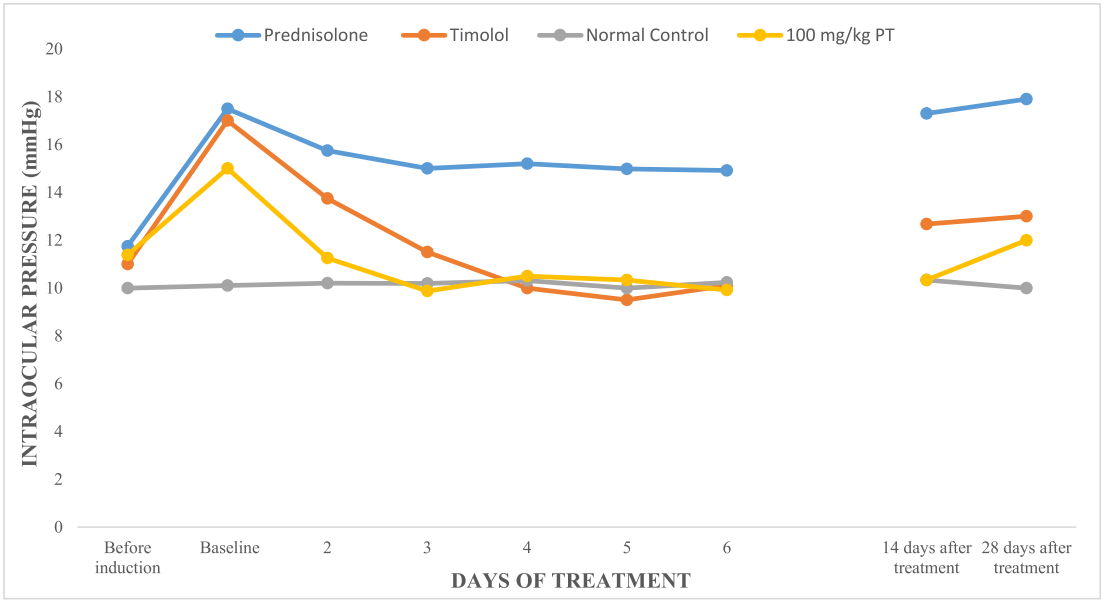

Fig. 6. Effect of Pleurotus tuber-regium *in ocular pressure at lowest doses of treatment.*

and poly-chemicals. *P. tuber-regium* or its synergic combination with other plants elicited cure for a wide range of ailments, include; headaches, stomach pain, fever, colds, nervous disorders, asthma, and constipation [22,23]. It has also validated in the management of hypertension, diabetes, hyper-triglyceridemia, fungal and bacterial infections, and tumors [24–27]. The objectives of this study were to investigate *P. tuber-regium* with preliminary qualitative and quantitative phytochemical screening, non-enzymatic antioxidants and its reducing effect on intraocular pressure in rabbits.

2. Materials and methods

2.1. Plant collection and identification

Pleurotus tuber-regium (oyster mushroom) was cultivated at the African Centre for Mushroom Research and Innovation (ACMRIT) domiciled in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, for 6 months (April–Sept). At maturity, they were harvested by the Mycologist [28–31]. A herbarium voucher number was issued (UBH-W731).

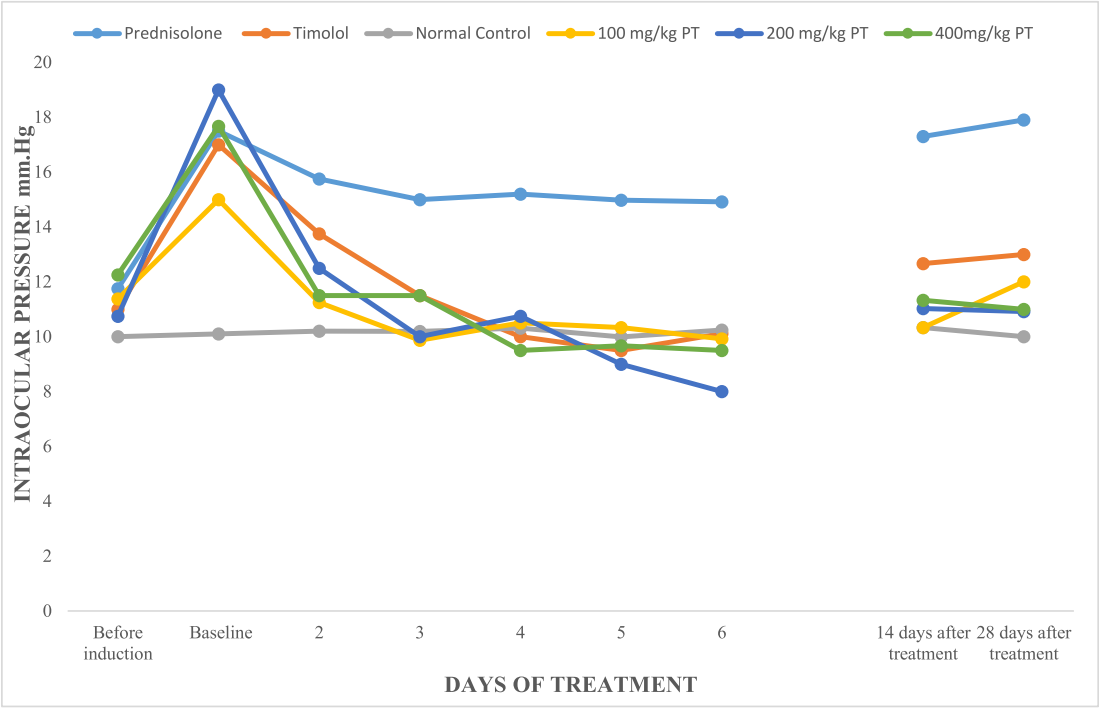

Fig. 7. Effects of Pleurotus tuber-regium *graded doses on intraocular pressure (IOP).*

2.2. Plant preparation and extraction

Pleurotus tuber-regium was cut into pieces, shade dried for three weeks, pulverized into powdery forms using a British milling machine, and stored in an air-tight container. The dried sample was subjected to a cold maceration technique using aqueous solvent system. This involved soaking the weighed powdered sample (1200 g) into a jar diluted with distilled water (2500 mL) for 72 h with intermittent steering and shaking. The filtrate was then fixed and dried in a freezer, and a semi-solid extract of the concentrate was produced and refrigerated for further use.

2.3. Phytochemical screening

This process involved the qualitative and quantitative analysis of the phytochemical properties of *Pleurotus tuber-regium* (oyster mushroom) [32,33].

2.4. Quantitative phytochemical analysis

This was done using the methods described by Sofowora [34], Trease and Evans [35], Muraleedharannair et al. [36], and Daniel et al. [37]. The extract was analyzed to identify the qualitative and quantitative phytochemical constituents present include; alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, saponins, and glycosides.

2.5. Antioxidant assay

This study involved the analysis of the antioxidant assay using the following methods described by Buchner et al. [38], Büttner et al. [39], and Cai et al. [40].

2.5.1. Total antioxidant capacity

One (1) mL of extract (1 mg/ml) was added to 3 ml of Molybdate reagent solution in tubes and incubated at 95 °C for 90 min. After which, the tubes were left for 20–30 min s to cool to room temperature, and the absorbance of the mixture was measured at 695 nm. Ascorbic acid was used as the standard [38].

2.5.2. Diphenyl-2-picrylhydrazyl (DPPH)

The free radical scavenging capacity of the leaf extracts against 1, 1–diphenyl–2–picrylhydrazyl (DPPH) radical was determined by a slightly modified method by Büttner et al. [39]. The mixture after was measured spectrophotometrically at 517 nm. Briefly, 0.5 mL of 0.3 mM DPPH solution in methanol was added to 2 mL of various concentrations (0.2–1.0 mg/mL) of the extracts. The reaction tubes were shaken and incubated for 15 min at room temperature in the dark, and the absorbance was read at 517 nm. All tests were performed in triplicate. Ascorbic acid was used as a standard control with similar concentrations as the test samples were

prepared. A blank containing 0.5 mL of 0.3 mM DPPH and 2 mL methanol was prepared and treated as the test samples.

2.5.3. Ferric reducing antioxidant power (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) assay was carried out using a modified method of Cai et al. [40]. The assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2, 4, 6-tripyridyls-triazine (TPTZ), forming an intense blue Fe^{2+}- TPTZ complex with an absorption maximum at 593 nm. To 1.5 mL of freshly prepared FRAP solution (25 mL of 300 mM acetate buffer pH 3.6, 2.5 mL of 10 mM 2,4,6-tripyridyls-triazine (TPTZ) in 40 mM HCl, and 2.5 mL of 20 mM ferric chloride($FeCl_3.6H_2O$) solution was added to 1 mL of the extracts (1 mg/ml) and standard at concentrations of 100–600 μM. The reaction mixtures were incubated at 37°C for 30 min, and the increase in absorbance at 593 nm was measured. FeSO4 was used for the calibration curve, and ascorbic acid served as the positive control. FRAP values expressed as (mg Fe (II)/g) of the extracts were then extrapolated from the standard curve [41].

2.6. Experimental animals

The experiment involved thirty (30) healthy adult New Zealand rabbits and ninety-five (95) healthy albino rats of Wister strains, purchased from Aduwawa market in Benin-city, Edo state, Nigeria. They were housed in the Department of Biochemistry, animal house, University of Benin. They were acclimatize for two weeks with free access to Bendel pelleted grower mesh and water *ad libidum*. They were exposed to 12 h of light and darkness. Their care conformed to the standard Ethical Right Guidelines for experimental animals. Life Sciences Ethical Committee certified the use of animals for this study with the ethical number LS19346.

2.7. Experimental design

This study consisted of thirty (30) healthy adult New Zealand rabbits randomly divided into six groups (n = 5); normal control, standard drug (0.5% timolol), untreated control (1% Prednisolone acetate suspension), and graded doses of treatment groups (100, 200 and 400 mg/kg) of *P. tuber-regium* respectively [42]. Before the study, the external eye tissues of the animals were screened with a penlight and hand magnifier. The internal eye examination was done with a direct ophthalmoscope for possible complications. The baseline intraocular pressure was obtained using a Perkins hand-held tonometer every morning before the study for 3 days to determine the average reading [43]. All the animals except those in the normal control were induced with ocular hypertension using topical corticosteroid (1% Prednisolone acetate) suspension. The application was administered daily in the right eye (OD) only for 7 days, and the IOP measurements were taken until the IOP reading increased by >5–10 mm Hg above the baseline readings [44].

2.8. Statistical analysis and data presentation

The statistical analysis was performed using the statistical package for Graph-pad prism version 7. The results obtained for the quantitative phytochemical screening, *in-vitro* antioxidant, and intraocular pressure were expressed as Mean ± SEM. One-way analysis of variance (ANOVA) test was used to determine the significant differences between the treatment groups.

3. Results

The phytochemical properties screened include; the presence of alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, saponins, and glycosides, as well as quantitatively determining their various concentrations. Phytochemical screening of *Pleurotus tuber-regium* mushroom extract showed the presence of flavonoids, phenols, tannins, and saponin were found as shown in Table 1.

The aqueous extract elicited a scavenging effect against oxidative stress, possibly subjecting the eye to stress, which could trigger eye disorders. It scavenge free radical, thereby had a significant decrease in percentage inhibition of DPPH, Total antioxidant capacity, and Ferric reducing antioxidant capacity. Antioxidants can donate hydrogen atom that neutralizes free radicals and changes the absorption process calorimetrically.

The results from 100, 200, and 400 mg/kg of *P. tuber-regium* elicited a significant decrease in intraocular pressure levels. At 200 and 400 mg/kg of the

Table 1. Preliminary qualitative and quantitative phytochemical properties of Pleurotus tuber-regium *(PT).*

Phytochemicals	Qualitative	Quantitative
Constituents	*Pleurotus tuber-regium*	*Pleurotus tuber-regium*
Alkaloid	–	0.0%
Flavonoids	+	1.29%
Phenols	+	20 mg/100 g
Tannins	+	28 mg/100 g
Saponins	+	5.90%
Glycosides	–	0.0%
Terpenoids	–	0.0%

Keys: – undetected, + detected.

mushroom extract, it exhibited a significant reduction in the mean IOP level when compared with 0.5% timolol and 1% Prednisolone acetate suspension. More so, the result obtained from *P. tuber-regium* aqueous extract exhibited a prolonged half-life and possibly triggered a substained ocular-hypotension when compared with timolol.

4. Discussion

The phytochemical properties screened include; the presence of alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, saponins, and glycosides, as well as quantitatively determining their various concentrations. Phytochemical screening of *Pleurotus tuber-regium* mushroom extract showed the presence of flavonoids, phenols, tannins, and saponin were found as shown in Table 1. This present study is similar to the work of Rajurkar and Kunda [45], Agati et al. [46], whose study is centered on the phytochemicals and metal content evaluated in *Adiantum capillus − veneris* extract. These compounds are active against potentially significant diseases [37]. Apart from their potential biological activity, the phytoconstituents such as saponins, flavonoids, terpenoids, phenols, and tannins, elicited anti-malaria, immunoprotection, anti-diarrhea, anti-ulcer, nociceptive, antihypertensive, anti-diabetes, anti-lipidemia, and anti-depressant. The phenolic compound potentiated the regenerations of blood cells which is an effective anti-anemic agent [47]. The number of phytochemicals found in *Pleurotus tuber-regium* was determined using standard procedures, and the result exhibited different quantities of phytochemical constituents. Tannin content is the highest in *Pleurotus tuber-regium* extract at 28.00 mg/100 g, followed by phenol compounds in *Pleurotus tuber-regium* at 20.00 mg/100 g in the extract. This study agreed with the preliminary phytochemicals screening reported by Kumudhavalli, and Jaykar [48] that evaluate petroleum ether, chloroform, acetone, ethanol, and aqueous fern extracts *Hemionitis arifolia*. The evaluated mushroom extract revealed the major phytoconstituents such as phenol, alkaloids, flavonoids, tannins, saponins, and terpenes compounds. This report is in line with the research work done by Muraleedharannair et al. [36], which examined the phytoconstituents of *Adiantum caudataum, Adiantum latifolium, Adiantum lunulatum, Christella dentate,* and *Christella parasitica* extracts.

The oxidation of ferric reducing antioxidant power reduces the presence of hydrogen-donating antioxidant capacity, thereby impelling antioxidant capacity in the duration of the reaction during antioxidant activity. The evaluation of total antioxidant capacity indicated higher antioxidant content of *Pleurotus tuber-regium* aqueous extracts at different concentrations (10, 20, 40, 60, 80, and 100 μg/ml). This present study agreed with the work of Yildiz et al. [49] on some physicochemical characteristics, bioactive content, and antioxidant capacity of non-sprayed Barberry (*Berberis vulgaris* L.) fruits from Turkey. The scavenging effect of the extract was recorded at higher concentration. This phenomenon was observed when measuring the antioxidant activity of the 50% ferric reducing antioxidant scavenging effect. The free radical scavenging effect was measured as percentage inhibition in the case of stable ferric reducing antioxidant properties at 50.0, 50.0, and 55.0% of the extract when compared with the 95.0% ascorbic acid result. The report concurred with Tupe et al. [50] that worked on the antioxidant potentials and total phenolic contents of selected Indian herbs powder extracts. The standard drug (vitamin C) elicited high percentage inhibition at a reduce concentration when compared with the extract. Since ascorbic acid is water-soluble, it serves as a potent antioxidant property to enhance the therapeutic effect on biological system [51].

Free radical scavenging effect evaluated as percentage inhibition of *P. tuber-regium* aqueous extract at diverse concentrations to scavenge DPPH radicals at about 100% when compared with 98% ascorbic acid at the same concentration. Hence, the inhibitory percentage increase is adequate with an increase in the concentration of *P. tuber-regium* extract. This, therefore, concurred with the possibility of the mushroom extract being capable of donating an electron to a lipid radical by converting the ascorbate radical to terminate the lipid peroxidation chain reaction. *P. tuber-regium* extract elicited a reduction in DPPH radical in line with hydrazine when reacted with hydrogen donors.

The results from 100, 200, and 400 mg/kg of *P. tuber-regium* elicited a significant decrease in intraocular pressure levels. At 200 and 400 mg/kg of the mushroom extract had a better significant decrease in the mean IOP level when compared with 0.5% timolol and 1% Prednisolone acetate suspension. More so, the result obtained from *P. tuber-regium* aqueous extract exhibited a prolonged half-life and possibly triggered ocular-hypotension when compared to 0.5% timolol. The action of endogenous carbapol polymer aid in the drainage of aqueous humor competence to cause an increase in intraocular pressure by exciting the trabecular meshwork [52]. Hence, a decrease in intraocular pressure amounts to the endogenous release of Neostigmine, causing an increase in acetylcholine via inhibiting

the action on Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE). This exhibit some exogenous or endogenous mediators capable of altering the frontal or anterior chamber angle, thereby instigating the primary inflammatory reaction with late stringy changes. This excitatory feedback action ensued in the early phase with reduction or total extinction after some weeks [53]. Fibrous disintegration and linkage in the barrier of the frontal chamber. Thus, the trabecular meshwork with normal lymph showed an extended and deformed eye caused by prednisolone administration, leading to collagen hyperplasia and the elastic fibers. The separation of the endothelial cells from the trabecular meshwork is comparable to that of the macrophages [54]. During the induction of intraocular pressure with prednisolone suspension, the phagocytized carbomer elements were elated via vacuoles in the Schlemm's canal of the endothelial chambers. The large vacuoles progressively decreased, leading to extreme carbomer particles amassed in the carbapol polymer, affecting the discharge of aqueous humor capability via the distorted trabecular meshwork. This present study evaluates the IOP lowering effect of *Pleurotus tuber-regium* in rabbits when compared with untreated IOP [5,44,55].

At graded doses of (100, 200, and 400 mg/kg) *P. tuber-regium* resulted in a significant decrease in normal intraocular pressure levels. At 200 and 400 mg/kg of the mushroom extract had a significant reduction in the mean IOP when compared with timolol control and untreated control. This agreed with the report of Liang et al. [56]. The result obtained from *P. tuber-regium* mushroom extract exhibited a delayed onset of action and possible triggering ocular-hypotension when compared with timolol. The possible IOP lowering effect of *P. tuber-regium* could be linked with the action anticholinesterase by reducing the excessive secretion of acetylcholine. This is in line with a previous study by Ji et al. [57] that worked on the effect of elevated IOP on mouse ganglion. Proofs have been suggested that gluco-corticoids are implicated in the metabolism of carbohydrates, fats, and protein, displaying their main role in a physiological directive of the pathway associated with the outflow capacity and intraocular pressure. Glucocorticoid receptors in trabecular meshwork pathway enhance aqueous outflow, biochemical, and resultant physical processes, competence in instigating reductive action. The mechanisms of action may be consequential to the modulatory action of macromolecular in adrenergic/prostaglandin interfaces encompassing the outflow system. Prednisolone triggered fibrous proliferation destroys the anterior chamber [58,59]. The eye is surrounded by several muscarinic receptors and their subtypes implicated in the ocular surface, lens, retina, ciliary body, and sclera.

5. Conclusion

In conclusion, *Pleurotus tuber-regium* extract is a potent therapeutic regime for managing intraocular pressure in rabbits. Scientific validation carried out affirms the ethnomedicinal claim that it is effective in the management of ocular hypertension.

Funding

Not applicable for this section.

Statement & declarations

We did intend to submit our manuscript to your reliable journal, and a copy of this manuscript has not been in any way under consideration or published elsewhere. No issue concerning the Journal competing interest. All authors have agreed to the publication of this manuscript.

Informed consent

Not applicable for this section.

Data availability statement

Data obtained from this study were presented as Tables and Figures. The materials used for this study, such as; chemicals, medicine, kits, and experimental animals, were procured standard stores within and outside the country.

Acknowledgments

Our earnest gratitude goes to Dr. Agu for the antioxidant assays in the Department of Medical Biochemistry, University of Benin, and Mycofarm research institute, Benin City.

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