SPECTROSCOPIC AND CHROMATOGRAPHIC CHARACTERISATION OF MUSHROOM EXTRACT (*Pleurotus tuberregium*) IN COMPARISON WITH SOME ANTIGLAUCOMA MEDICATIONS (0.5% TIMOLOL AND 0.005% LATANOPROST)

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ABSTRACT

Pleurotus tuberregium, an edible fungus, occurs in both tropical and subtropical regions of the world. Scientific evidences exist for the use of *P. tuberregium* in the treatment of high blood pressure, diabetic hypertriglyceridemia, fungal and bacterial infections, tumours and raised intraocular pressure. However, its comparative chromatographic and spectroscopic analysis with anti-glaucoma medications has not been extensively explored. This study separated the bioactive constituents of the mushroom extract and compared it with 0.5% Timolol and 0.005% Latanoprost. Fractions of the extracts were obtained through Column chromatography utilizing silica gel. Retardation factors and migration speeds of the fractions were then obtained using Thin Layer Chromatography. UV-VIS spectrophotometry was then utilized to obtain a more refined result. The experiment produced comparative retardation factors and retardation factors of the extracts with those of the antiglaucoma medication. Spectroscopic studies on the extract revealed that it has an absorption spectrum within the ultra violet wavelength range with a λ_{max} of 320nm. All spots for this study were produced with a reproducibility factor better than 1.5% RSD.

Keywords: Anti-glaucoma medications, Bioactive Constituents, Column Chromatography, *Pleurotus sp.*, Spectrophotometry, Retardation Factor.

INTRODUCTION

Glaucoma, as a term, encompasses a diverse group of disorders which have in common a characteristic potentially progressive optic neuropathy that is associated with visual field loss as damage progresses, and in which intraocular pressure is a modifiable factor [1]. The first port of call in the management of glaucoma is the use of anti-glaucoma medications. However, the cost of glaucoma medications as well as their side effects has been reported as some of the major deterrents to drug adherence and compliance. These complaints can be effectively attended to by herbal remedies. Several nutrients and botanicals hold promise for the treatment of glaucoma, but most studies are preliminary, and larger, controlled studies are required [2].

Several edible mushrooms have been reported to contain pharmacological active ingredients such as flavonoids, tannins, etc. [3]. Muhammed reported that mushrooms heal eye diseases [4]. The gilled basidiomycete, *Pleurotus tubberegium*, found in Nigeria has been attributed with numerous medicinal properties [5

– 8]. Ocular hypertensive cats, treated with extracts of *Pleurotus tubberegium* experienced significant decrease in intraocular pressure [9]. Similarly it has been documented that *Pleurotus tubberegium* was used in the treatment of headache, stomach pain fever, cold, constipation [10]. This study evaluated the active agents in extracts of *Pleurotus tubberegium* and compared them with those found in selected anti-glaucoma medications.

MATERIALS AND METHODS

Column Chromatography and Thin layer Chromatography were carried out at the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, while UV-VIS Spectrophotometry was carried out at the Spectrophotometry/Chromatography center at the National Center for Energy and Environment (NCEE), University of Benin, Benin City, Edo State.

The experiment was executed in four phases. During the first phase, fresh *P. tuberregium* mushrooms were cultivated and extracts were obtained using water as a solvent. The selected

anti-glaucoma drugs to be used in the study were basified with ammonium hydroxide to render them preservative free. In phase two, the extracts were separated into fractions using a 100ml long column loaded with silica gel and hexane and acetone as diluting solvents. Here, a total of thirty 10ml fractions were collected and monitored using TLC. Fractions with similar constituents were pooled together in a properly labeled test tube. During Phase three, freshly prepared Thin Layer plates were manually spotted with the obtained fractions, 0.5% Timolol and 0.005% Latanoprost, and then

placed in an iodine tank. Photographs were then taken and migration speeds and retardation factors measured. Retardation factor (Rf) values were calculated using the following formula:

$$R_f = \frac{\text{Distance Travelled by Solute}}{\text{Distance Travelled by Solvent}} \quad [11].$$

In the final stage, the analytes were further analyzed with a Spectrophotometer at the National Centre for Energy and Environment. The absorption spectrum obtained were compared.

RESULTS

The weight and appearance of the five fractions obtained from Column Chromatography were tabulated thus:

Table 1: Weight and appearance of fractions from Column chromatography.

No.	Weight (g)	Test-tubes	Appearance
A1	0.281	1-2	Brownish-oil
A2	0.657	3-6	Brownish-oil
A3	0.817	7-15	Yellowish-oil
A4	0.513	15-21	Colourless viscous structure.
A5	No spot	22-30	Colourless

A5 was chemically empty, which is why it produced no spots.

Two readings were taken and the mean of their migration distances and retardation factors

recorded and presented in figures and bar charts below.

Figure 1: Thin layer chromatography (TLC) plate 1 showing separation spots of samples A1, A2 and A3.

Figure 2: Thin layer chromatography (TLC) plate 3 showing separation spots of 0.5% Timolol.

Figure 3: Thin layer chromatography (TLC) plate 4 showing separation spots of samples preservative free 0.5% Timolol and fractions (A1, A2, A3 and A4) of 40mg/ml *Pleurotus tuberregium* extract. T = 0.5% Timolol.

Retardation factor was calculated by dividing migration distance of the sample with the migration distance of the solvent. The migration

distance of the solvent for plates 1 to 5 was 12cm.

ANOVA**Table 2:** Table showing analysis of variance between groups and within groups.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.110	3	.037	2934.653	.014
Within Groups	.000	1	.000		
Total	.110	4			

Table 3: Table showing One Sample statistics of A1, 2% Pilocarpine, 0.5% Timolol, 0.5% Betaxolol and 0.005% Latanoprost.

	N	Mean	Std. Deviation	Std. Error Mean
A1	2	4.00850	4.796305	3.391500
TIMOLOL	2	4.00300	4.789941	3.387000
LATANOPROST	2	4.44150	5.315322	3.758500

Table 4: Table showing the results of the student one sample t-test in comparing the means between the groups.

One-Sample Test						
	Test Value = 7.40					
	T	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
0.5% Timolol	-1.003	1	.499	-3.397000	-46.43292	39.63892
0.005% Latanoprost	-.787	1	.575	-2.958500	-50.71477	44.79777

From the analysis of variance above, we can conclude that there are similarities of migration

distance and retardation factor between fraction A1 and 0.5% Timolol.

Figure 4: A plot of the absorbance spectrum of A1
A1 recorded a wavelength of maximum absorption (λ_{max}) of 280nm.

Figure 5: A plot of the absorbance spectrum of A2 at varying wavelengths

A2 recorded a wavelength of maximum absorption (λ_{max}) of 220nm.

Figure 6: A plot of the absorbance spectrum of A3 at varying wavelengths
A3 recorded a wavelength of maximum absorption (λ_{max}) of 320nm.

Figure 7: A plot of the absorbance spectrum of A4 at varying wavelengths.

DISCUSSION

The retardation factor of Latanoprost discovered by this study is 0.686. This is concurrent with the findings of a monograph by the United States Pharmacopeia, which stated that the retardation factor of Latanoprost is about 0.7 [12]. The migration distance and retardation

factor of A1 on the Thin Layer Chromatogram (7.30) was found to closely approximate that of 2% Pilocarpine and 0.5% Timolol, 7.33 and 7.41 respectively. This finding suggests that there is a possible existence of the active ingredients of these antiglaucoma medications in A1. This concurs with the study on Bovine Iris Sphincter

muscle where it produced a contractile effect on the muscarinic receptors of the sphincter papillae and its effect on the intraocular pressure of hypertensive cat eyes [13 – 14].

The migration distance and retardation factor of fraction A2 and 2% Pilocarpine were discovered to be relatively similar. Latanoprost a prostaglandin analogue was reported by a monograph by the United States Pharmacopoeia as having an absorption spectrum within the range of 200 – 400nm, with a λ_{max} of about 200nm. This study discovered the Rf value of Timolol to be 0.62. Another study placed the Rf value of Brimonidine and Timolol at 0.23 and 0.63 respectively. The accuracy of the method of the stated study was accessed by percentage recovery and found to be $99.77 \pm 0.71\%$ for brimonidine tartrate and $99.87\% \pm 0.86\%$ for Timolol maleate. This study also concluded that the TLC method can be used for routine analysis of brimonidine tartrate and timolol maleate in pharmaceutical formulations [15]. However, the European Pharmacopoeia method for Timolol Identification Test C using silica gel G TLC plates reported the Rf value of Timolol to be 0.74 [16]. The disparity in value between this study and that is a result of the type of silica gel plates used.

A1 recorded a wavelength of maximum absorption (λ_{max}) of 280nm. A2 recorded a wavelength of maximum absorption (λ_{max}) of 220nm. A3 recorded a wavelength of maximum absorption (λ_{max}) of 320nm. A4 recorded a wavelength of maximum absorption (λ_{max}) of 320nm. This connotes that the maximum absorbance wavelength of the extract is 320nm. Timolol maleate, a non-selective beta blocker reduces has an absorbance spectrum that falls within this range with a λ_{max} of 295nm as determined by past research [17]. Though not a drug under comparison in this study, Brimonidine tartrate, a relatively selective α_2 adrenergic receptor agonist whose mechanism of action is to reduce aqueous humour production and increase uveoscleral outflow has a λ_{max} of 247nm [18].

The fractions of the *Pleurotus tuberregium* extract demonstrated corresponding spots to those of the antiglaucoma drugs used in this study. This suggests a possible existence of a combination of similar active ingredients in the

extracts and the drugs. This could explain its intraocular pressure reducing properties [9]. It is clear that the absorption spectrum of *Pleurotus tuberregium* falls within the range of the absorption spectra of the antiglaucoma drugs under comparison. It is therefore very likely that the mushroom extract has similar active ingredients as the drugs under study and hence similar pharmacodynamics activity.

CONCLUSION

Research has shown that *Pleurotus tuberregium* has diverse medicinal applications. Experimental studies on feline eye models and isolated bovine iris have proven some of its acclaimed properties and activities. A chromatographic study on this mushroom species in comparison with other common and effective anti-glaucoma medications is pertinent. This study successfully compared the separation spots and retardation factors of fractions of *Pleurotus tuberregium* extracts with those of drugs whose mode of action and pharmacodynamics are already known. That effective of *Pleurotus tuberregium* in reducing intraocular pressure has been proven. How it achieves this feat however is yet to be properly documented. There is a need to determine its composition and concentration of bioactive compounds to correlate it with pharmacological activities. This can be achieved by comparative studies with other drugs. The information obtained from this study can serve as a bedrock for further research in the discovery of a less harmful and more pocket friendly anti-glaucoma therapeutic.

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