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Phytochemical and Toxicological Properties of Sclerotium from the Edible Fungus - *Pleurotus tuber-regium*

Chinwe S. Alaribe[1]*, Mariam Lawal[1], Rashidat Momoh[1], Ufoma Idaholo[1], Pamela Mudabai[1], Abdullahi A. Adejare[2]

[1]*Dept of Pharmaceutical Chemistry Faculty of Pharmacy, University of Lagos, Nigeria*
[2] *Dept of Physiology, Faculty of Basic Sciences, College of Medicine, University of Lagos, Nigeria*

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ABSTRACT

Edible fungi such as mushrooms have been known to possess great potentials in the production of useful bioactive metabolites for drugs and nutraceuticals. The aim of this study was to investigate the phytochemical constituents present in sclerotium from *pleurotus tuber regium* and to determine its safety dose using animal model toxicity assay.
Phytochemical tests used for preliminary evaluation of the secondary metabolites present in sclerotium was carried out on the n-hexane and 50 % ethanol extracts while the n-hexane extract was subjected to Gas-chromatographic-Mass spectrometric (GC-MS) techniques for the identification of the compounds present. Acute toxicity studies were carried out on aqueous methanol extract of sclerotium using standard *in vivo* model. Hematological and histopathological parameters were evaluated.
Phytochemical analysis results showed the presence of alkaloids, steroids in the n-hexane extract and the presence of cardenolides and steroids in the 50% ethanolextract. GC-MS results showed the presence of nine compounds; Squalene, Dibutyl phthalate, Octadecane, Heneicosane, 9, 17 octacadienal, 2-octyl-cyclopropaneoctanal, carbonic acid, Bis(2-ethylhexyl) phthalate, Nonacosane. The toxicity studies showed no death at the highest dose (5000 mg/kg) administered, although moderate portal and lobular inflammation were observed.
The present study has shown that the Sclerotium from the edible fungus - *Pleurotus tuber-regium* is not toxic to experimental mice at the highest dose of 5000 mg/kg. Hence, the extract is relatively safe when administered orally.

Keywords: *Edible Fungus,* sclerotium, *pleurotus tuber-regium, GC-MS,toxicity*

Introduction

Safety in the use of Natural products is as important as their other benefits either as food, immune boosters, supplements or drugs. Nigeria as a nation is blessed with numerous natural products of plant, animal and marine sources with rich medicinal properties. One of such is an edible gilled fungus, *Pleurotus tuber regium* (Fr.) Singer, a Basidiomycete belonging to the Pleurotaceae family and commonly known as the King Tuber Mushroom. Historically, *Pleurotus tuber-regium* (Fr.) is found in tropical regions including Equatorial Africa, Australia, Asia and other areas in the South Pacific but was first collected in Africa by Elias Magnus Fries which was later described and reclassified by Rolf Singer in 1951.[1] Aside the facts that they are nutritiously consumed as food, Mushrooms have a very long folkloric use in Nigeria with amazing medicinal properties. In addition to the significance of this fungus, *Pleurotus tuber-regium* (Fr.) is their fruiting body called sclerotium, a storage tuber and underground mass of mycelia often produced in a unique manner as the fungus consumes wood. At present, it has been reported that between 80 and 85% of all medicinal mushroom fungi are derived from their fruiting bodies. Other isolates from fungi fruiting bodies include; lentinan from *Ganoderma lucidum*, PSK and PSP from *versicolor*, tremellastin from *Tremella mesenterica*, scutigeral from *Scutiger ovinus* (Schaeff: Fr.).[2]
Sclerotia are a compact mass of mycelia tissue that serves to store food for fungus *Pleurotus tuber regium* during unfavorable conditions and are able to fruit when favorable conditions return. They are usually dark brown on the surface, white inside, spherical to ovoid in shape and are as large as 30 cm in diameter.[3,4] Their pharmacological and nutritional benefits cannot be overlooked. It has been reported that many compounds such as B-D-glucans, non-starch polysaccharides and enzymes such as ribonuclease with potential pharmacological benefits are present in the sclerotium of *Pleurotus tuber-regium*.[5] Sclerotia are used in various soup and medicinal preparations both for human consumption and in traditional medical practice in Nigeria.[6]
In China, sclerotium is used in some folk recipes as a tonic and medicine for the treatment of coughs and asthma.[7] In South Eastern Part of Nigeria, sclerotium, a fiber-rich tuber is used as soup thickener because of its ability to swell in water and add bulk. It has also been reported to be useful in the treatment of heart problems, asthma, cough and obesity.[8,9] Other folkloric uses include treatment of headache, stomach ailments, colds, cough, fever and smallpox.[10]
World Health Organization (WHO) has been stressing the need for total involvement of traditional systems of medicine to meet the goal "Health for All" and its call for integration of the Nigerian and other medicinal herbal products into health systems by 2023.[11] However, identifying secondary bioactive metabolites, standardization and establishing the safety profiles of these resourceful natural products either crude or finished product have been a challenge in this part of the world. This limitation initiated the investigation of the phytochemical contents of sclerotium extracts and their toxicity profile. More so the fact that the toxicological studies were also evaluated following the perception that

*Corresponding author. Email: aalaribe56@gmail.com;
salaribe@unilag.edu.ng
Tel: +234-8037263962

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reasonable number of fungi and related products are poisonous especially if they were to be used as food and Medicine. *Haematologica* ,

Materials and Methods

Fungi (sclerotium) collection and Preparation
The sclerotia of *Pleurotus tuber regium* were purchased from Oyingbo market in Lagos State, Nigeria between February and March, 2015 and taxonomically identified by Mr. Oyebanji of the Department of Botany, University of Lagos with voucher number "LUH: 003M". The sclerotia were washed several times to remove dirt and debris. The free debris sclerotia were then cut into smaller pieces and air-dried. The dried sclerotia were then milled into powdery form and stored for further use.

Fungi extraction for qualitative studies
The powdered sclerotia (500 g) were subjected to cold maceration successively with n-hexane and 50% ethanol (EtOH) for 7 days. The extract was filtered and the filtrate was oven dried at 45°C and the mass of extracts in grams was recorded.

Fungus preparation and extraction for toxicological studies
Approximately 500 g of the powdered sclerotium was weighed and soaked into 1.5 L of 50% Methanol (MeOH) for 96 hours after which it was filtered. The filtrate was collected and concentrated using rotary evaporator (BUCHI) at 45ºC. A stock solution, 250 mg/mL was prepared from the aqueous methanol extract and kept in the refrigerator prior to administration.

Phytochemical screening
Phytochemical tests for the presence of secondary metabolites; Cardiac glycosides, Anthraquinone Glycosides, Alkaloids, Steriods, Tannins (Hydrolysable and condensed), Flavonoids, Saponins, Reducing sugar were carried out on both n-hexane and 50% Ethanol extracts of sclerotium using standard procedures.[12,13]

Gas Chromatography – Mass Spectrometric analysis of n-hexane sclerotia extract
Separation and spectroscopic analysis were carried out on *n*-hexane sclerotia extracts using GC-MS (Agilent Technologies) equipped with HP-5ms capillary column (Length: 30.0 m, Diameter: 0.25 mm, film thickness : 0.25 µm composed of 100 % Dimethyl poly siloxane). The GC-MS had an electron ionization system with ionization energy of 70 eV. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. The instrument was set to an initial temperature of 110ºC and maintained at this temperature for 2 min. At the end of this period, the injector and the interface temperature were set at 250ºC and 280ºC, respectively at an increasing rate of 5ºC/min and maintained for 9 min. The samples were injected in split mode as 10:1 and the mass spectral scan range was set at 45- 450 (m/z). Interpretation of mass spectra (GC-MS) was done by using data base of National Institute of Standards and Technology (NIST,2016).

Experimental animals
Albino male and female mice aged 5-8 weeks of average weight 20 g were used for this study. The animals were acclimatized for 7 days and were provided with feed and water. Handling of animals was done in accordance with international acceptable guidelines and approval from College of Medicine, University of Lagos Ethical Committee with reference number; CMUL/HREC/03/17/099.

Acute toxicity assay
A total of 20 mice were used and distributed into four groups (I – IV) of five mice each. Group I (5000 mg/g); Group II (2500 mg/g); Group III (1000 mg/g); Group IV (control, only feed and water). The animals were subjected to 24 h of fasting prior to the experiment and the doses given orally using an orogastric tube. After 24 h, two animals, randomly selected in each treatment groups were sacrificed, and their blood collected by eye puncture into labeled sample bottles for haematological analysis and their organs (liver, kidneys and heart) were excised and transferred into sample bottles containing 10% formalsaline, a combination of formaldehyde and normal saline for histopathology test. These processes were repeated for the remaining animals after 14 days of treatment.

Haematological assessment of aqueous methanol extracts of Sclerotium
The Coulter Haemalogy method similar to Soulaf Jabbar Kakel.[14] was used in this study. The haematology analyzer (Mindray BC-3200) operating at ambient temperature was used to perform a complete blood count. Whole blood (13 µL) was aspirated; a diluent, about 3.5 mL consisting of an isotonic electrolyte solution was used to dilute the whole blood samples and to also stabilize cell membranes for accurate counting. Lyse, a lytic reagent was also used to separate red blood cells from the white blood cells before counting. The analyzer calculates the Mean Corpuscular Volume (MCV), Haematocrit (HCT), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

Histopathological assessment of aqueous methanol extract of sclerotium
Preparation of tissue for micrometry
Preparing tissues for micrometry which included grossing or cutting up, tissue processing, embeddling were carried out. For grossing or cutting up, the tissues were cut into thin slices of about 0.3 cm – 0.5 cm thickness with a scalpel blade and placed in embedding cassettes ready for processing. The tissues processing involved cutting of the tissues through several reagents enabling tissue consistency to be suitable for micrometry. 10% formal-saline was used as a fixative, grades of alcohol from 70% alcohol to absolute alcohol were used as dehydrates, xylene was used to treat the tissues in clearing after dehydration which was removed in the process of impregnation. After the tissue was completely impregnated with wax, a solid block containing the tissues was obtained. This was achieved by filling a mould of suitable size with molten paraffin wax, orienting the tissue in the centre of the mould to ensure its being cut in the right plane and finally cooling the wax to promote solidification. Rotary microtomy was used to obtain sections from tissues.

Haemotoxylin and eosin staining technique
The sections obtained were de waxed and taken to water. The specimen was stained with Cole's haemotoxylin for 10 mins and rinsed in blue warm water for 1-3 mins. It was afterwards stained in 1% eosin for 3 mins and rinsed in water. The specimen was dehydrated in alcohol and cleared in xylene. The specimen was mounted with DPX and colour change observed. Blue was taken as the nucleus while pink was taken as the cytoplasm.

Results and Discussion

Human health is at the moment been challenged by rapid urbanization, globalization of unhealthy lifestyles, uncertainty of climatic changes as well as adverse effects of agents synthetically prepared in form of drugs to manage disease conditions. In this present decade, the world is resorting to the use of natural products in the management of different ailments. However, their standardization and safety qualities are of paramount importance.
The phytochemical screening of the n-hexane extract of Sclerotium of *Pleurotus tuber-regium* revealed the presence of alkaloids and steroids, and the absence of flavonoids, reducing sugar and carbohydrate while the 50% ethanol extract revealed the presence of cardenolides, and steroids. The Gas Chromatographic - Mass Spectrometric analysis of the n-hexane extract of sclerotia identified nine compounds (Table 1 and Figure 1). The compounds include; Squalene, a dehydrotriterpenic hydrocarbon with a retention time of 23.946 min; Dibutyl phthalate, a dibutyl ester oily liquid and an endocrine disruptor with retention time of 18.421 min; Octadecane, an alkane hydrocarbon, with a retention time of 18.760 min, Heneicosane with a retention time of 18.930 min; 9,17-Octadecadienal, an aldehyde; with a retention time of 20.708 min; Cyclopropaneoctanal,2-octyl with retention time of 21.008 min; Carbonic acid-decyl-undecyl-ester having retention time of 22.923 min; Phthalate, a diester of phthalic acid with retention time of 25.847 min and Nonacosane, straight-chain hydrocarbon with retention time of 27.171 min. The presence of these phytochemicals and even more could be responsible for its medicinal prowess. [15,16]
Haematological toxicity assay afforded the information on the safety quantity of sclerotia to be consumed per time. A great number of users

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Table 1: GC-MS analysis results of n-hexane extracts of sclerotium from *Pleurotus tuber-regium*

Peak	Retention time (mins)	Peak height	Area	% Total in abundance	Compound
1	18.421	1273727	31268216	6.783%	Dibutylphthalate
2	18.760	5724442	60596494	13.145%	Octadecane
3	18.930	677381	71473609	15.504%	Heneicosane
4	20.708	113938	11460378	2.486%	9,17-Octadecadienal, Z
5	21.005	46097	3103473	0.673%	Cyclopropaneoctanal, 2-octyl-
6	22.923	83007	14059936	3.050%	Carbonic acid, decylundecyl ester
7	23.946	147583	12344751	2.678%	Squalene
8	25.847	2567564	141611400	30.718%	Bis(2-ethylhexyl) phthalate
9	27.171	958106	115079194	24.963%	Nonacosane
		Total	460997451	100%	

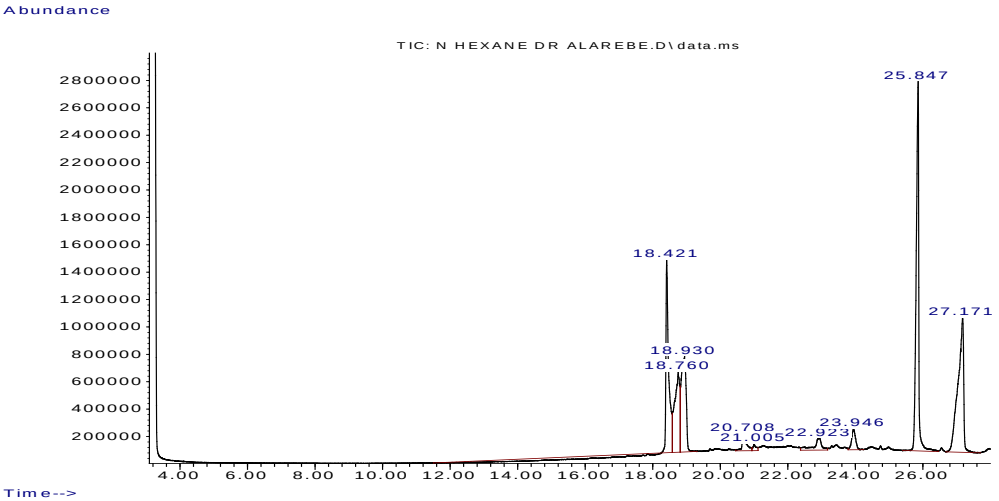

Figure 1: GC chromatogram of the n-hexane extract of the sclerotium of *Pleurotus tuber-regium*.

utilize this edible fungal product without adequate precaution on its toxic effect possibly with time. For this assay, diluted lysed blood samples were used for easy identification, passage through the aperture one at a time as well as to create a conductive environment for cell counting. Haematology results of aqueous methanol extracts of sclerotia revealed that there was a significant increase in the white blood cells (WBC) after 24 h of increasing doses of the extracts compared to the control group as shown in table 2. The increase in WBC could be a way of the body responding to what is alien to it. The results also revealed significant decrease in Red Blood Cells (RBC), Haematocrit (HCT), and Hemoglobin (HGB) in all the three groups compared to control indicating anemic conditions.

A slight increase in Mean Corpuscular Volume (MCV) was also observed in group 1 while groups 2 and 3 showed significant increase in MCV when compared with control group. An increase in MCV could be as a result of impending liver challenges due to overload of this extract.

For Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC), a slight decrease was observed between group 1 while significant increase between groups 2 and 3 when compared with control group. Decrease in MCH and MCHC has been associated with loss of blood, iron deficiency anemia and spherocytosis signifying the presence of spherocytes, a type of RBCs that contain abnormally high amount of haemoglobins in the blood. Also, an increase in MCH is an indication of macrocytic anemia, a blood disorder where limited and abnormal numbers of red blood cells are produced.

On the other hand, Haematology results after 14 days of administration showed little or no significant change as when same extracts was administered for only 24 h (Table 3).

The histopathology results showed no changes in the heart and kidney at the highest dose of 5000 mg/g administered for 24 h and 14 days monitoring compared with the control group. However, there were significant changes in the liver in form of mild lobular inflammation on the same dose of 5000 mg/g when compared with the control group at 24 h and 14 days administration. The haematology and histology findings recommend caution on the use of sclerotium by patients with anaemic and hepatic impairment conditions.

Conclusion

The safe use of natural products as food or drug is paramount especially if utilized for a considerable period of time. Sclerotium, a fungal product and one of such natural products subjected in this study constitutes as food condiments and serve as agents to alleviate cardiovascular and other diseases. Phytochemical assay identified alkaloids and steroids in the n-hexane extract while cardenolides, alkaloids and steroidal nucleus were also confirmed present in the 50 % ethanolic extract. GC-MS results showed the presence of nine compounds while toxicity studies showed zero death at the highest dose (5000 mg/kg) administered though moderate portal and lobular inflammation were observed hence caution is recommended on its use.

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Table 2: Haematological results after 24 hours of administration of n-hexane sclerotia extracts

Blood cells		Extract		
	Control	1000 mg/g	2500 mg/g	5000 mg/g
WBC ($X10^9$/L)	6.4 ± 0.25	7.5 ± 0.15*	7.8 ± 0.11*	10.2 ± 0.18*
RBC ($X10^{12}$/L)	8.57 ± 0.18	8.35 ± 0.27	7.88 ± 0.15*	7.28 ± 0.16*
HGB (g/dL)	13.9 ± 0.22	13.2 ± 0.26*	12.9 ± 0.21*	12.4 ± 0.14*
HCT (%)	39.1 ± 0.71	38.4 ± 0.78	35.3 ± 0.87*	32.9 ± 0.93*
MCV (f/L)	45.7 ± 0.61	46.1 ± 0.65	44.9 ± 0.58	45.2 ± 0.62
MCH (ꝑ/L)	16.2 ± 0.63	15.8 ± 0.74	16.3 ± 1.01	17.0 ± 0.84
MCHC (g/dL)	35.5 ± 0.49	34.3 ± 0.67*	36.5 ± 0.46*	37.6 ± 0.62*

*P<0.05 when compared with the control group.

Table 3: Haematology results after 14 days of administration of n-hexane sclerotia extracts

Blood cells		Extract		
	Control	1000 mg/g	2500 mg/g	5000 mg/g
WBC ($X10^9$/L)	5.5 ± 0.11	7.9 ± 0.08*	7.7 ± 0.15*	9.3 ± 0.25*
RBC ($X10^{12}$/L)	9.84 ± 0.15	9.1 ± 0.17*	9.67 ± 0.31	8.75 ± 0.34*
HGB (g/dL)	14.6 ± 0.20	13.5 ± 0.14*	14.7 ± 0.23	13.2 ± 0.12*
HCT (%)	47.2 ± 0.78	42.4 ± 0.87*	49.0 ± 0.88*	42.6 ± 0.78*
MCV (f/L)	48.0 ± 0.58	46.7 ± 0.62*	50.7 ± 0.64*	48.7 ± 0.45
MCH (ꝑ/L)	14.8 ± 0.28	14.8 ± 0.17	15.2 ± 0.09*	15.0 ± 0.12*
MCHC (g/dL)	30.9 ± 0.57	31.8 ± 0.66	30.0 ± 0.62	30.9 ± 0.49

*P<0.05 when compared with the control group.

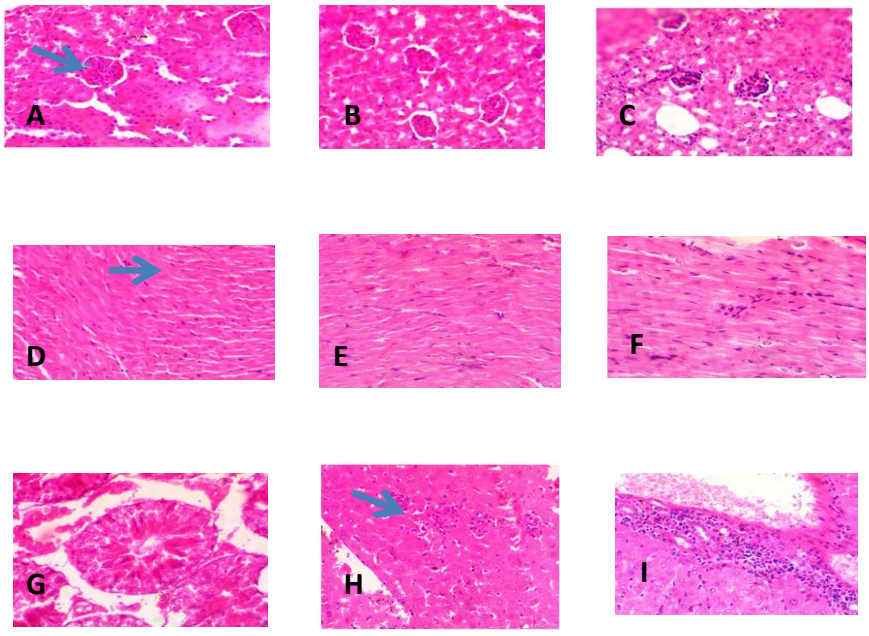

Figure 2: Histopathology views of aqueous methanol extracts of sclerotium in three selected organs; Kidney, Heart and Liver.

Notes: **A**: Kidney control group. arrow showing glomerulus **B**: Kidney group 1, 5000mg/g after 24hrs; No changes seen **C**: Kidney group 1, 5,000 mg/g after 14 days; **D**: Heart control group; arrow showing cardiac myocytes. **E**: Heart group 1, 5000mg/g after 24hrs; No changes seen. **F:** Heart, group 1, 5000 mg/g after 14 days; No changes seen. **G**: Liver control group - No liver tissue seen. **H**: Liver, group 1, 5000 mg/g after 24 Hrs; arrow showing mild lobular inflammation. **I**: Liver, group 1, 5000mg/g after 14 days with portal and lobular inflammation.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Huang HY, Mallikarjuna K, Chaing YY, Chien TY, Tsai YC. *Pleurotus tuber-regium* polysaccharides attenuate hyperglycemia and oxidative stress in experimental diabetic rats. J Evid Based Comp Alt Med. 2012; 7:856381.
2. Ulrike L, Timo HJN, Wolf-Dieter J. The Pharmacological Potential of Mushrooms. J Evid Based Comp Alt Med. 2005; 2(3): 285.
3. Okhuoya JA and Okogbo FO 'Cultivation of *Pleurotus tuber-regium* (Fr) Sing on Various Farm Wastes'. Proc Okla Acad Sci. 1991; 71:1-3.
4. Oso BA. *Pleurotus tuber-regium* from Nigeria. Mycologia. 1997; 60: 271-279.
5. Akpaja, EO, Isikhuemhen, OS, Okhuoya, JA. Ethnomycology usage of edible and medicinal mushrooms among the Igbo people of Nigeria. Int J Med Mushrooms 2003; 5:313-319.
6. Zoberi MH. Some Edible Mushrooms from Nigeria. Nigerian field 38. 1973; 81-90.
7. Chenghua D, Xiangliang Y, Xiaoman G, Yan W, Jinggyan Z, Huibi X. A ß-D-glucan from the sclerotia of *Pleurotus tuber-regium* (Fr.) Sing. Carbohydr. Res. 2000; 328:629-633.
8. Isikhuemhen OS, Okhuoya JA. A low cost technique for the cultivation of *Pleurotus tuber-regium* (Fr) singer, in the developing tropical countries. Mushroom Growers Newsletter. 1995; 4:24.
9. Isikhuemhen OS, Osemwegie OO, Onyolu, Okhuoya JA. Cultivation of a selected sporophore-only-producing strain of edible and medicinal mushroom, *Pleurotus tuber-regium* (Fr) Singer (Agaricomycetideae) on waste paper and plantain peeling. Int J Med. Mushrooms. 2002; 4:343-348.
10. Iwuagwu, AM, Onyekweli, OA. Preliminary investigation into the use of *pleurotus tuber-regium* powder as a tablet disintegrant. Trop J Pharm Res. 2002; 1(1):29-37.
11. World Health Organization. Traditional Medicine Strategy (2014–2023), WHO, Geneva, Switzerland. 2013.
12. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria. 1993. 289 p.
13. Trease GE, Evans WC. Drugs of biological origin, In: Pharmacognosy, 12th ed. United Kingdom Balliere Tindall; 1989. 309 – 540 p.
14. Soulaf. JK. The evaluation of traditional and automatic Coulter method in estimation of haematological parameters in adult rats. Beni-Suef University J. Basic and Applied Sci. 2013. (2):31-35.
15. Farvin KHS, Anandan R, Kumar SHS. "Cardio- protective effect of squalene on lipid profile in isoprenaline- induced myocardial infarction in rats," J Med Food. 2006; 9(4):531–536.
16. Kelly GS. "Squalene and its potential clinical uses," Alt Med Rev. 1999; 4 (1):29–36.