IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 13, Issue 11 Ser. I (November. 2019), PP 16-20
www.iosrjournals.org

Determination of the Phytochemical Properties of Oil Extracted From Meat of *Python Bivittatus*

[11]Offurum, J.C.; [2]Chukwu M.M.; [3]Mbadike C.A.; [4]Nwakaudu A.A.; [5]Iheme C.
[1,2,3,5]Department of Chemical Engineering, Imo State Polytechnic, Umuagwo-Ohaji.
[4]Department of Food Science Technology, Federal University of Technology, Owerri.

Abstract: *This research work focused on the determination of the phytochemical properties of oil extracted from the meat of nonvenomous snake specie, 'Python Bivittatus', a Burmese Python, which is considered as sub-specie of the Python Molurus. Varying forms of snake (python) oils exist, with varying characteristics, each having different concentrations/strengths of activity. The python oil used for this study was collected from the extraction point at Umunnochi in Isukwuato L.G.A. of Abia State of Nigeria, where the dealers employed local (smoking) techniques to separate the oil from the meat of the already-dead python. The oil was subjected to centrifugation to remove inherent particulate matters. Phytochemical analysis (qualitatively and quantitative) were conducted on the oil sample, using the standard stipulated by the Association of Official Analytical Chemist (1997) as revised. The results of qualitative analysis showed that there is a little presence of alkaloids, tannis, and cardiac glycosides (+) in the sample; phenols indicated moderate presence (++), while saponins, Terpenoids, steroids and flavonoids showed heavy presence in the sample (+++). The quantitative analysis showed that tannins, saponins, alkaloids, flavonoids, phenol, steroids, terpenoids and cardiac glycosides were 61 .43%, 16.32%, 0.72%, 467.65µg/ml, 0.160mg/g, 5.91mg/100g, 206.08mg/100mg 82.40% respectively. The results, generally, show that the study sample is well established with phytochemical characteristics, which amongst other several values (especially medicinally), makes it a very viable fluid for corrosion control and mitigation.*

Keyword*: Analysis, Python Oil, Corrosion Control, Metallic Material*

Date of Submission: 24-10-2019 Date of Acceptance: 09-11-2019

I. INTRODUCTION

Snakes are legless scaly creatures (vertebrates) that are found in almost every biome. They are among the most feared animals on the planet. While some species of snake are born with two heads, others can reproduce without males (Bailey, 2018). Okoli *et al* (2018) reported that there are over 3,000 snake species in existence, ranging the 4-inch Barbados thread-snake to the 40-feet Anaconda snake. According to the report, snakes can slither, swim and/or glide through the air (an act that is better explained as flying). Python Oils are oils extracted from the meats/fats of pythons. Though more than 40 species/sub-species of python exist, only a few of them are usually hunted for the oil values. The present attention given to python oils is traceable to the Chinese ancient traditional use of the oil for medicinal purposes. Slanshinski *et al* (2012) reported that Chinese water-python oil contains up to 20% eicosapentaenoic acid (EPA), one of the two types of omega-3 fatty acids, most readily used on human bodies. The report further reviewed that Salmon (one of the most popular food sources of omega-3) contains a maximum of 18 percent EPA, which is lower than that of the python oil; omega-3s are vital for human metabolism. Not only do they sooth inflammation in muscles and joints, they can also help for cognitive functions and reduce blood pressure, cholesterol, and even depression.

Phytochemicals, on the hand, are chemical compounds that occur naturally in plant and animals. They are basically produced by the host for self-protection against environmental threats, such as predators, pollution and disease. Harbone (1998) reported that thousands of distinct phytochemicals are derived from carotenoids and polyphenols, some of which are non-essential food nutrients, and are, thus, not required to sustain life. Beyond metabolic functions of phytochemical parameters as disease inhibitors, they also inhibit metallic corrosions.

This study, therefore, focuses on the determination of phytochemical properties of oil extract from the meat of *'Python Bivittatus'* of the *Pythonidae* family, sub-specie of the *Python Molurus*, basically for its use as inhibitor against metallic corrosion.

Theoretical Extracts On Species Of Python

As initially mentioned, more than 3,000 species of snake exist, from which more than 40 species/sub-species of members of the pythonidae family were derivable. Some of the species of python, as reviewed by Schleip and O'Shea (2010) are as follows:

Malayopython Recticulatus: Recticulated python; also in the pythonidae family. It is native to South and Southeast Asia. It is listed as the world's longest specie of snake. It is usually hunted, in several regions, for its skin, for use in traditional medicine and for sale as pet.

Python Genus: Constricting snake in the pythonidae family; native to tropics and subtropics of the eastern hemisphere.

Python Molurus: Popularly known as Indian python. It is black tailed, and considered as third largest snake specie.

Python Bivittatus: A Burmese python, that is nonvenomous, found in Southern Asia, Africa and Australia. This class is among the five largest specie of snake. It is considered as sub-specie of python molurus. It has beautiful patterns, docile and used as pet. Members of this class are recognized world's old snake.

African-Rock Python: It is found in Sub-saharan Africa. It is considered as sub-specie of the python genus.

Python Curtus: It is commonly known as Sumatran short-tailed python. It is native to Sumatra of the Western Indonesia.

Timor Python: It is commonly found in Southeastern Asia. It is dwarfed, and not considered dangerous to humans.

Borneo python: It is a nonvenomous python that is epidemic to island of Borneo.

Python Brongersmai: It is a short-tailed python, red-colored and nonvenomous. It is native to Malay Peninsula of the Thailand Emirate.

Python anchietae: It is a nonvenomous python; it is endemic to South Africa, and related to Angolan dwarfed python.

II. Materials And Methods

The python oil was sourced from the dealers at Umunnochi in Isukwuato L.G.A. of Abia State of Nigeria. The collection was at the point of extraction, for the sake of unwarranted adulteration. It was then subjected to further purification by means of centrifuge. Centrifugation was conducted batch-wise at the speed of 100rpm within 10minutes, using activated carbon. Using filter paper, filtration was conducted, to separate the particles-in-adsorbent and the purer oil (filtrate). The prepared sample was then subjected to phytochemical screening using the standard stipulated by the Association of Official Analytical Chemist, AOAC (1997).

2.1 Qualitative Analysis of Phytochemical Parameters

Test for Tannins: 0.5g of the oil sample was boiled in 20ml of distilled water in a test tube, after which it was removed (at boiling) and filtered into a conical flask by means of a filter paper. 0.1% $FeCl_3$ was then added to the filtrate and a reddish colour change was observed, which indicated the presence of Tannin.

Test for Saponins: 0.5g of the sample was boiled with 20ml of distilled water (in a water bath), and was removed (at boiling) and then filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously, to obtain stable persistent froth. The frothing was then mixed with 3drops of olive oil and observed for the formation of emulsion; the formation of emulsion indicated the presence of saponin.

Test for Alkaloids: 0.5g of the test samples was treated with four (4) drops of potassium mercuric iodide solution (Dragendroff's reagent), and a cream colouration was observed, which indicated the presence of Alkaloid.

Test for Cardiac Glycosides: 1ml of concentrated H_2SO_4 was prepared in a test tube. Then 5ml of the oil extract was mixed with 2ml of glacial acetic acid (CH_3CO_2H), containing 1drop of $FeCl_3$. The mixture was carefully added to the 1ml of concentrated H_2SO_4 so that the concentrated H_2SO_4 was underneath the mixture; observation for a brick-red precipitate was made, which indicated the presence of cardiac glycoscides.

Test for Terpenoids: 5ml of extract was mixed in 2ml of chloroform, and concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the inner face was observed, indicating the presence of terpenoid.

Test for Streroids: 0.5ml of the chloroform was added into a test tube containing 5ml of extract. Then 1ml of concentrated H_2SO_4 was carefully added to form a lower layer. The colour at the interface, which indicated the presence of steroids, was observed and noted.

Test for Phenols: 5g of the sample was boiled with 2ml of phenol in a water bath, and then filtered. 2ml of the filtrate was pipetted into a conical flask, and 10ml of distilled water was added. A brownish colour was observed, indicating the presence of Phenol.

Test for Flavonoids: 3drops of 1%-ammonia (NH_3) solution was added to the sample in a test tube, and a pale-yellow precipitate was observed, which indicated the presence of Flavonoid.

2.2 Quantitative Analysis of Phytochemical Parameters

Percent Tannin Determination: 20g of the sample was put into a conical flask, and 100ml of petroleum ether was added and covered for 24hrs. The mixture was then filtered and exposed for 15minutes, to allow traces of the petroleum ether to evaporate. It was then soaked in 100ml of 10%-acetic acid in ethanol for 4hrs, after which it was filtered. 25ml of NH_4OH solution was added to the filtrate to precipitate the alkaloids, which were heated in electric hot plate to remove any traces of NH_4OH still in the solution. The remaining volume was noted, from which 5ml of it was taken and combined with 20ml of ethanol. The mixture was titrated with 0.1M solution of sodium hydroxide (NaOH), using phenolphthalein as indicator. Appearance of pink colour indicated the end point. The tannin content is given by equation 1.

$$\text{The tannin content, } C_1 = \frac{C_2 V_2}{V_1} \qquad 1$$

Where, C_2 = Molar Conc. of NaOH = 0.1M

V_1 = Vol. of Ethanol = 5ml

V_2 = Vol. of NaOH = Titre Value; and equation 1(a) was used to obtain the percentage tannic acid content.

$$\%\ \text{Tannin Content} = \frac{C_1\ \text{X}\ 100}{\text{m}} \qquad 1(a)$$

Where m = Mass of sample analysed.

Percent Saponin Determination: 5g of the sample was weighed into a beaker containing 20%-acetic acid (in ethanol), and allowed to stand in a water bath, at 50^oC, for 24hrs. This was filtered, and the filtrate was concentrated using a water bath to one-quarter of its original volume. Concentrated NH_4OH was then added drop-wise to the mixture until precipitation was completed. The whole solution was allowed to settle, and the precipitate was collected (through filtration) and weighed. The saponin content (expressed in percentage) is given by equation 2.

$$\%\ \text{Saponin Content} = \frac{m_2\ \text{X}\ 100}{m_1} \qquad 2$$

Where m_1 = mass of Sample analsed

m_2 = mass of residue (after filtration)

Percent Alkaloid Content Determination: 5.0g of each of the sample was weighed into a beaker, and 200ml of 10% acetic acid (in ethanol) was added to the beaker. The mixture was covered and allowed to stay for 4hrs, after which it was filtered; the filtrate was concentrated by means of water bath to one-quarter of the original volume. Then concentrated ammonium hydroxide solution (NH_4OH) was added drop-wise until precipitation was completed. The whole solution was allowed to settle and the precipitate was collected, washed with dilute NH_4OH and then filtered. The residue (alkaloid), was dried in an oven, weighed and percent weight was evaluated and recorded.

Percent Cardiac Glycoside Content Determination: 1ml of the test sample was added to 1ml of 2%-solution of 3,5-Dinitro Salicylic Acid (DNS) in methanol and 1ml of 5%-aqueous NaOH. It was boiled for about 2minutes (until a brick-red colour was observed), and the boiled sample was filtered (having taken note of the weight of the filter paper used). The absorbed residue was then dried to dryness in an oven at 50^oC. The weight of the dried residue was evaluated and noted accordingly. The percentage cardiac glycoside content is given by equation 3.

$$\%\ \text{Cardiac Glycoside Content} = \frac{m_2\ \text{X}\ 100}{m_1} \qquad 3$$

Where m_1 = mass of Sample analysed

m_2 = mass of residue (after drying to dryness)

Terpenoid Content Determination: 5ml of sample was pipetted into the test tube and put into a sizeable beaker, and 2ml of chloroform was added to it. Then 0.1ml of H_2SO_4 (Sulhuric acid) was added. The terpeniods content was read on the calibration curve and noted. The blank solution was prepared using the same procedure. The terpeniods content was determined using equation 4.

$$\text{Terpeniods Content, } \frac{\text{mg}}{100\text{g}} = \frac{\text{Conc. of standard X Absorbance of Sample}}{\text{Absorbance of Standard X Weight of Sample}} \qquad 4$$

Steroid Content Determination: 1ml of methanolic extract of steroid solution was transferred into volumetric flask containing 10ml of sample. 2ml sulphuric acid and Iron (ii) chloride (0.5% $^{w}/_{v}$ 2ml) were then added, followed by potassium hexacyanoferrate (iii) solution (0.5% $^{w}/_{v}$, 0.5ml). The mixture was heated in a water bath maintained at 70^0C for 30minutes, with serial shaking and dilution to the maximum calibration point, using

distilled water. The absorbance was measured (at 780nm) and noted. The procedure was repeated for the blank. Steroid content was determined by equation 5

$$\text{Steriod Content}, \frac{\text{mg}}{100\text{g}} = \frac{\text{Conc. of standard X Absorbance of Sample}}{\text{Absorbance of Standard X Weight of Sample}} \qquad 5$$

Phenol Content Determination: The quantity of phenol in the sample was determined using the spectrophotometric method. 2g of the test sample was combined with 50ml of ethanoic acid and allowed to boil for 15minutes. Then 5ml of the boiled sample was pipetted (after cooling) into a 50ml conical flask, and 10ml of distilled water was added to it. Also 2ml of NH_4OH and 5ml of concentrated pentanol were added to the mixture. After thorough mixing of the reagents, the sample was made up to the reference mark of the flask and allowed for 30minutes for possible colour change; it was then collected and viewed in a U.V. spectrophotometer *(at 505nm wavelength)*. The reading represents the phenol content, and was noted accordingly (in mg of solution per 100g of sample).

Percent Flavonoid Content Determination: 10g of sample was put into a beaker, containing 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered (by means of filter paper), and the filtrate was transferred to a water bath and evaporated to dryness. The residue was then weighed and noted accordingly. The flavonoid content (expressed in percentage) is given by equation 6.

$$\%\ \text{Flavonoid Content} = \frac{m_2\ \text{X}\ 100}{m_1} \qquad 6$$

Where m_1 = mass of Sample analysed

m_2 = mass of residue (after evaporation)

III. Results And Discussion

The results the qualitative and quantitative analyses of the python oil sample are presented in table 1.

Table 1: Results of Phytochemical Screening of Sample

Parameter	Qualitative Analysis Result	Quantitative Analysis Result
Tannin Content (%)	+	61.43
Saponin Content (%)	+++	16.32
Alkaloid Content (%)	+	0.72
Cardiac Glycocides (%)	+	82.40
Terpenoid Content (mg/100g)	+++	206.08
Steroid Content (mg/100g)	+++	5.91
Phenol Content (mg/100g)	++	0.160
Flavonoid Content (%)	+++	467.65

The results of qualitative analysis of the study sample show that the oil has heavy presence of saponin, terpenoid, steroid and flavonoids contents, while phenol is moderately present, and tannin, saponins and cardiac glycocides are mildly present. But qualitative screening could basically serve two purposes; one is for feasibility assessment for economic utilization of the reagents, to ascertain whether or not to proceed for quantitative determination, and the other is for predictive purposes and possible approximations. (Offurum *et al*, 2017). Considering the second purpose, it could be deduced that the study sample is very rich in saponin, terpenoid, steroid and flavonoid contents, when compared with other parameters.

On the other hand, results the qualitative analysis showed prominent levels of the quantities analyzed. The flavonoid, terpenoid and cardiac glycocides contents were found to be more prominent (in their values) than other factors analyzed; it has been reported that flavonoid, terpenoid and cardiac glycocide (and also steroid and alkaloid) are biogenic/defensive compounds that can serve efficient lubricating functions, alter ergosteroi functions of fungi, cause loss of function mutations in the enzymes, prevent lipid peroxidation for diseases and metal degradation and scavenging of free radicals (Kumari *et al*, 2014; Sparkman and Kitson, 2011; Vigan, 2010; Xuan *et al*, 2018). In view of these, the study sample could be considered a viable tool for corrosion mitigation. The presence of saponins in the study sample makes it useful for industrial production of detergents, commercial foam making (as stabilizer), and for use as emulsifying agent (Arivalagan, *et al*, 2013). Also, tannins and polyphenols respectively are natural pigments and flavouring substances, and as such could give the study sample industrial values in the production of dyes and beers/wines (Chai *et al*, 2018; Bele *et al*, 2010).

IV. Conclusion

Oil extract from the body (meat) of python bivittatus has proven to be well established in phytochemical properties such as flavonoid, terpenoid, cardiac glycocide, steroid, alkaloid, and so on. By these characteristics, it has, amongst other benefits, been shown to be a viable inhibitor for metallic corrosion. It is, therefore, presented for useful applications in our industries.

References

[1]. Arivalagan M., Gangopadhyay K.K. and Kumar G. (2013) **. Determination of Steroidal saponins and Fixed Oil Content in Fenugreek**; Indian Journal of Pharmaceutical Sciences; 75(1): 110-113.
[2]. Association of Official Analytical Chemist, AOAC (1997)**. Official Method of Analysis**; Alpha Books Inc.; Washington D.C.
[3]. Bailey R. (2018) **. 7 Weird Facts About Snakes**; Science Technical; 8(2): 181-188.
[4]. Bele A.A., Jadhav V.M. and Kadam V.J. (2010) **. Potentials of Tannins: A Review**; Asian Journal of Plant Sciences; 9(4): 209-214.
[5]. Chai F.K., Adzahan N.M., Karim R., Rukayadi Y. and Ghazali H.M. (2018) **. Characteristics of fat, Saponin and Tannin Contents of 11 Varieties of Rambutan Seeds**; International Journal of Food Properties; 21(1): 1091-1106;
[6]. Gakuubi M.M., Wagac E.O. Freire K.R. and Souza C.P (2005) **. Inhibitory Action of Some Essential Oils and Phytochemicals on the Growth of Various Moulds Isolated from Foods**; Brazilian Archives of Biology and Technology; 48(2): 245-250.
[7]. Harbon J.B. (1998)**. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis**; Chapman and Hall, New York.
[8]. Kumari S., Pundhir S. and Yadav G. (2014) **. A database of Essential Oils Reflecting Terpene**
[9]. **Composition and Variability in the Plant Kingdom**; Journal of Biological Database and Curation; 48(2): 96-103.
[10]. Offurum J.C., Nwakaudu M.S., Ndukwe O.C., Kamalu C.I.O. and Onyelucheya O.E. (2017) **.**
[11]. **Investigation of the Phytochemical Parameters in Ethyl-Esters of Rubber Seed Oils from**
[12]. **Imo Rubber Estate Obiti-Ohaji, Nigeria**; Continental Journal of Applied Sciences; 12(2): 22-32.
[13]. Okoli J.B., Adeyemo S.O. and Dele B.M. (2018) **. Physicochemical, Trace Metals Analysis and Fatty Acid Profile of Python Regius Fat**; Biotechnology journal; 4(10): 1064-1076.
[14]. Schleip W.D. and O'Shea M. (2010)**. Annotated Checklist of the Recent and Extinct Pythons (Serpentes, Pythonidae), with Notes on Nomenclature, Taxonomy and Distribution**; Australian Venom Research Unit, Pharmacology Department, University of Melbourne; Australia.
[15]. Slanshinski M.J., McCurdy S.A., Achenbaum L.S., Whitney S.N. and McGuire A.L. (2012)**. Snake Oil, Quack Medicine and Industrially Cultured Organisms: Biovalue and the Commercialization of Human Microbiome Research**; Centre for Medical Ethics; U.S.A.
[16]. Souza E.I., Lima E.O. Freire K.R. and Souza C.P (2005) **. Inhibitory Action of Some Essential Oils and Phytochemicals on the Growth of Various Moulds Isolated from Foods**; Brazilian Archives of Biology and Technology; 48(2): 245-250.
[17]. Sparkman O.D. and Kitson F.G. (2011) **. Comprehensive Analytical Chemistry**; Elsevier Books; New York.
[18]. Xuan T.D., Minh T.N. and Khanh T.D. (2018) **. Total Phenolic and Flavonoid content of Edible Oil Extracts**; Research Journal of Chemical Environment; 9(1): 40-46.
[19]. Vigan M. (2010) **. Essential Oils: Renewal of Interest and Toxicity**; Euro-Deratol; 20(2): 685-692.

Offurum, J.C. " Determination of the Phytochemical Properties of Oil Extracted From Meat of Python Bivittatus." IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 13.11 (2019): 16-20.