Vol. 2 No. 1, June

FUAM Journal of Pure and Applied Science

Available online at
www.fuamjpas.org.ng

An official Publication of
College of Science
Joseph Sarwuan Tarka University,
Makurdi.

FUAM Journal of Pure and Applied Science
© College of Science, Joseph Sarwuan Tarka University, Makurdi
https://fuamjpas.org.ng/
Vol.2. No. 1. June, 2022

Antioxidant Activities of Hexane, Ethyl acetate, Acetone and Methanol Extracts of *Newbouldia Laevis*

***O.A. Ushie, G.O. Egah, T.J. Aasegh, A.I. Ibrahim, B. Bako and N. Agyo**
Department of Chemical Science, Federal University Wukari, Nigeria
*Correspondence E-mail: afiushie@yahoo.com ushie@fuwukari.edu.ng

Received: 21/04/2022 **Accepted**: 16/05/2022 **Published online**: 17/05/2022

Abstract

The crude extracts (n-hexane, ethyl acetate, acetone and methanol) were obtained by cold maceration method by soaking 100 g of the sample in 250 mL hexane for 72 hours with frequent agitation. All the extracts inhibited DPPH radical in a concentration of 0.1 mg/L, 0.3 mg/L, 0.5 mg/L, 0.7 mg/L, and 0.9 mg/L and methanol extract of *Newbouldia laevis* producing highest inhibition (which was not significantly different from vitamin C (66.57, 67.41, 71.31, 76.60 and 85.52). Results have shown that *Newbouldia laevis* plant have the capacity to protect or inhibit damage induced by free radical species.
Keywords: DPPH, Extract, *Newbouldia laevis,* Medicinal plants, free radicals, extract EDDDPPH scavenging activity, antioxidant activities

Introduction

Medicinal plants are used in all over the world to treat different types of human and animal diseases. The therapeutic usage of indigenous plant products for ethno-medicinal and nutritional objectives has attracted scientists' curiosity, motivating them to look for bioactive compounds [1]. Medicinal plants possess essential food components such as carbohydrates, protein, and fat. These components are important for the human body's requirements and they are used in different physiological, metabolic and morphological activities [2].

Medicinal plants used in the traditional medicine are well-known significant sources of natural antioxidants. Medicinal plants-derived natural antioxidants, which are in the form of raw extracts and/or chemical constituents, are very efficient to block the process of oxidation by neutralizing free radicals [3]. It is also commonly accepted that medicines taken from plant products are safer than their synthetic counterparts; however, the toxicity profile of most medicinal plants have not been comprehensively assessed [4]. A immense quantity of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties [5]. As long as free radicals are balanced by the body's antioxidative defense system, the body is in healthy conditions [6]. The depletion or loss of antioxidant levels may lead to free radical-caused oxidative stress. Oxidative stress can cause cellular and tissue damages, DNA mutation, cancer etc [7]. Oxidative stress is among the major contributory factors in induction of many chronic and degenerative diseases includin g atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others [8]. Oxidative process is one of the most significant routes for producing free radicals in foods, drugs and even in living systems [9].

Newbouldia laevis have recently attracted research interest because it possesses antioxidant properties against a variety of physiologically relevant free radicals [10]. Some of the documented medical uses include in the folk treatment of fevers (including yellow fever), malaria, stomach ache, cough, sexually transmitted infections, skin infections, tooth ache, breast cancer, constipation, pain (pelvic pain in females, chest pain, ear ache), gonococcalorchitis, elephantiasis, sore feet, ulcer, epilepsy, convulsion, migraine, sickle cell anaemia, as a febrifuge, as a vermifuge, in female reproductive healthcare (fibroids, infertility, hemorrhage), as aphrodisiacs, eye problems, snake bites, wound healing, diabetes, arthritis, rheumatism and other inflammatory conditions [11] – [14], [11] Some of the folkloric uses of this plant have been scientifically validated. Pharmacological studies on extracts of different parts of *N. laevis* have revealed the antioxidant and free radical scavenging [15], antimicrobial and antimalarial , sedative and anticonvulsant [16], analgesic, antinociceptive and antiinflamatory [17], hepatoprotective [18], anticancer [19], uterine contraction [20], wound healing and antiulcer [21], antisickling [22], hypoglycemic [23] activities among others.

The plant kingdom still holds for many species of plants containing substances of medicinal value, which are yet to be revealed. *Newbouldia laevis*is one of the plants which have been used in traditional medicine for decade years. To the best of our knowledge little or no work has been done on the plant *N. laevis*, Nigeria. This work is designed to enrich the available scientific data on antioxidant activities of and scientific proof on the antioxidant properties of *N. .laevis* thereby contributing.

Materials and Methods

Collection and preparation of sample

The *Newbouldia laevis* leaves were collected from their natural habitat in Wukari Local Government Area of Taraba State, Nigeria and were air dried for two weeks; the dried sample was chopped and grounded into fine powder. The extracts of the leaves were prepared by soaking 100 g of the sample in 250 mL hexane for 72 hours with frequent agitation. The resulting mixture was filtered by gravity filtration and the filtrate was concentrated by evaporation using rotatory evaporator, kept in a vacuum oven over night at room temperature to remove all the solvent and weighed. The procedure was repeated on the residue using ethyl acetate, acetone and methanol sequentially in order of polarity. The extracts were stored in desiccators until required for testing.

Antioxidant assay using DPPH Assay (2, 2-diphenyl-1-picrylhydrazyl)

The radical scavenging activity of different extracts was determined by using DPPH assay according to [24] and [25]. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

Principle

2, 2- Diphenyl -1- PicrylHydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

(DPPH) + (H-A) ⟶ DPPH-H + (A)

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Reagent preparation

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100 mL of ethanol.

Working procedure

Different volumes of the extract were taken and made up to2 mL with methanol. The following concentrations of the extract were tested (0.1, 0.3, 0.5, 0.7, and 0.9 mg/L). Vitamin C was used as the antioxidant standard at concentrations (0.1, 0.3, 0.5, 0.7, and 0.9mg/L). 0.5 mL of 1mM of DPPH in ethanol was added to each of the sample solutions. A blank solution was prepared containing the same amount of methanol and DPPH. The sample solutions are incubated in the dark for 3ominutes before reading the absorbance at 517nm. The radical scavenging activity was calculated using the following formula:

$$\text{Inhibition } (\%) = \frac{A - B}{A} \times 100 \qquad (1)$$

Where A = Absorption of the blank sample without extract.
B = Absorption of the extract.

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (Bilos, 1958). About 0.1mM of DPPH in ethanol was prepared and 1ml of this solution was added to 3.0 mL of extract solution in ethanol at different concentrations (0.1, 0.3, 0.5, 0.7, 0.9mg/mL). Thirty minutes later, the decrease or increase absorbance was measured at 517nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The same experiment was carried out on ascorbic acid which is known antioxidant. All test and analysis were run in triplicate and the results obtained were averaged.

Results and Discussion

Table 1: Antioxidant Activity of Hexane, Ethyl acetate Acetone Methanol Extracts, and Vitamin C.

	Inhibition (%)				
Concentration (mg/L)	HE	EAE	AE	ME	VCS
0.1	71.74	57.45	81.68	75.16	82.23
0.3	70.81	61.80	77.02	73.91	82.66
0.5	67.39	63.29	75.78	73.91	82.28
0.7	57.14	69.25	72.67	71.74	82.03
0.9	49.07	73.29	70.49	68.63	82.81

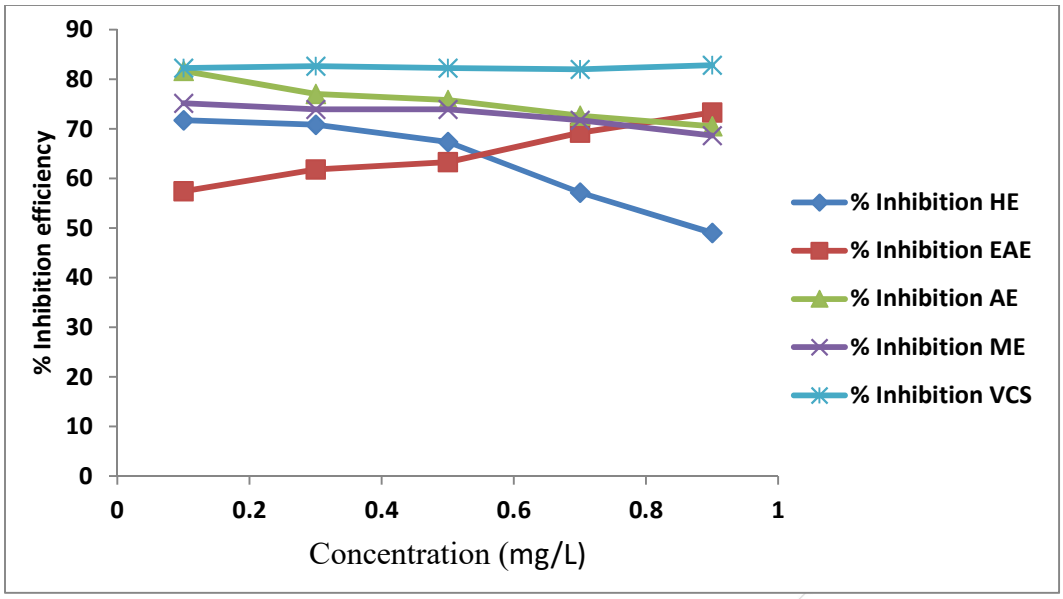

Figure 1: Plot of Concentration Vs % Inhibition for HE, EAE, AE & ME

To determine the antioxidant activity of a specific solution, there will be a significant decreased in the absorbance for sample which contain antioxidant compound (purple colour vanishing coupled with the yellow color build up clearly noticed by visual observation) the intensity of the yellow colour was directly proportional with the antioxidant activity in the tested solution, the higher scavenging indicate the higher activity [26] (Sagare and Singh 2011). The free-radical scavenging activity was evaluated by accessing it's discolouration of 2,2-diphenyl-1-picrylhrozyl radical (DPPH) in methanol by a slightly modified method. The following concentrations of the extract were tested (0.1, 0.3, 0.5, 0.7 and 0.9 mg/mL). The decrease in absorbance was monitored at 517nm. Vitamin C was used as the antioxidant standard at a concentration (0.1, 0.3, 0.5, 0.7 and 0.9 mg/mL). The crude hexane extract of *Newbouldia laevis* displayed inhibition of DPPH radical scavenging activity at the range of 49.07%, 57.14%, 67.39%, 70.81% and 71.74% with the concentration of 0.1, 0.3, 0.5, 0.7 and 0.9 mg/L respectively while vitamin C showed minimum radical scavenging activity of 82.03 % and maximum activity of 82. 66% (Figure1).

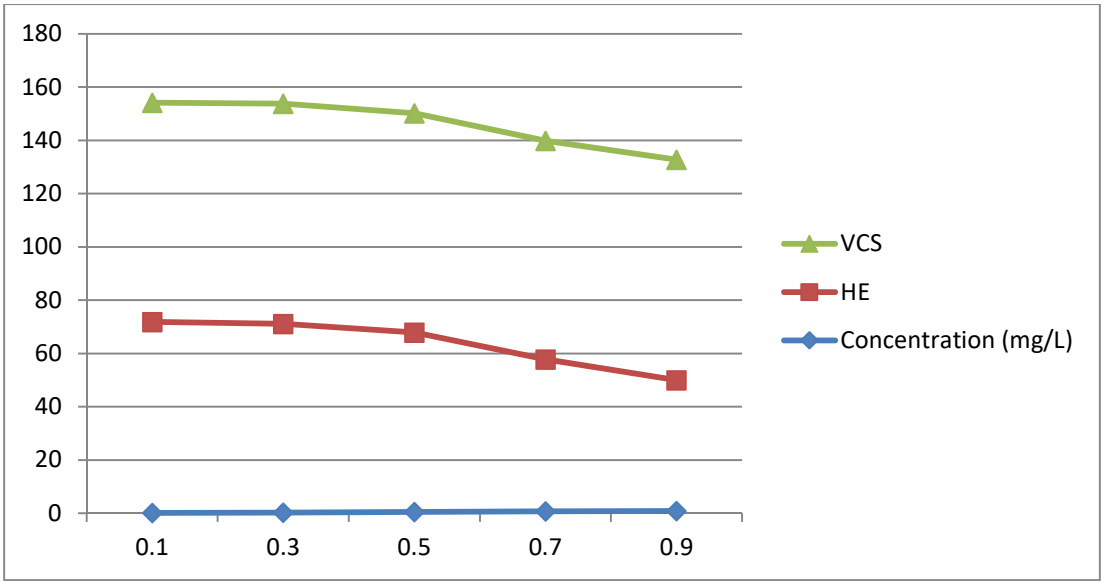

Figure 2: Plot of Concentration Vs % Inhibition for Hexane extract

The crude ethyl acetate extract of *Newbouldia laevis* displayed inhibition of DPPH radical scavenging activity at the range of 57.45%, 61.80%, 63.29%, 69.25% and 73.29% with the concentration of 0.1, 0.3, 0.5, 0.7 and 0.9 mg/L respectively while vitamin C showed minimum radical scavenging activity of 82.03 % and maximum activity of 82. 66% (Figure 2).

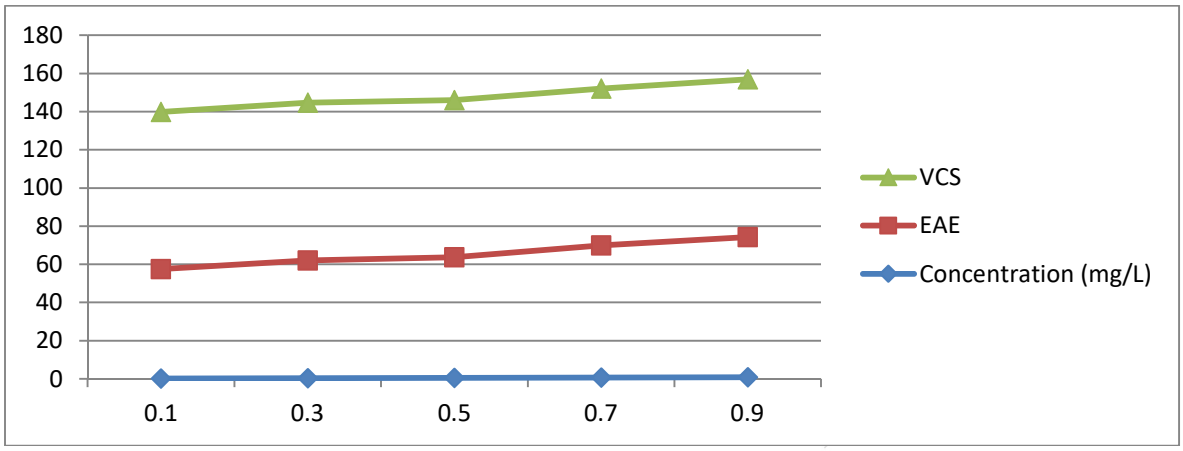

Figure 3: Plot of Concentration Vs % Inhibition for Ethyl acetate extract

The crude acetone extract of *Newbouldia laevis* displayed inhibition of DPPH radical scavenging activity at the range of 70.49%, 72.67%, 75.78%, 77.02% and 81.68% with the concentration of 0.1, 0.3, 0.5, 0.7 and 0.9 mg/L respectively while vitamin C showed minimum radical scavenging activity of 82.03 % and maximum activity of 82.66% (Figure 3).

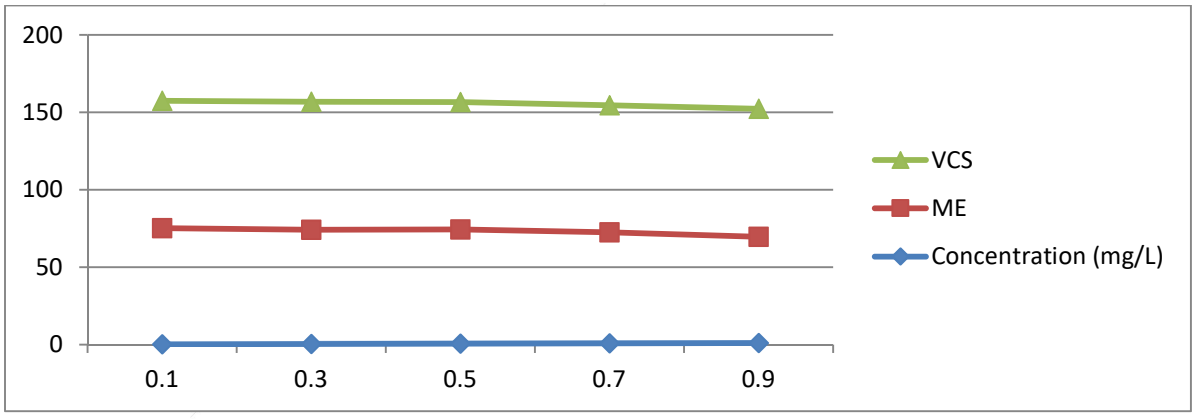

Figure 4: Plot of Concentration Vs % Inhibition for Acetone extract

The crude methanol extract of *Newbouldia laevis* displayed inhibition of DPPH radical scavenging activity at the range of 68.63%, 71.74%, 73.91%, 73.91% and 75.16% with the concentration of 0.1, 0.3, 0.5, 0.7 and 0.9 mg/L respectively while vitamin C showed minimum radical scavenging activity of 82.03 % and maximum activity of 82.66% (Figure 4).

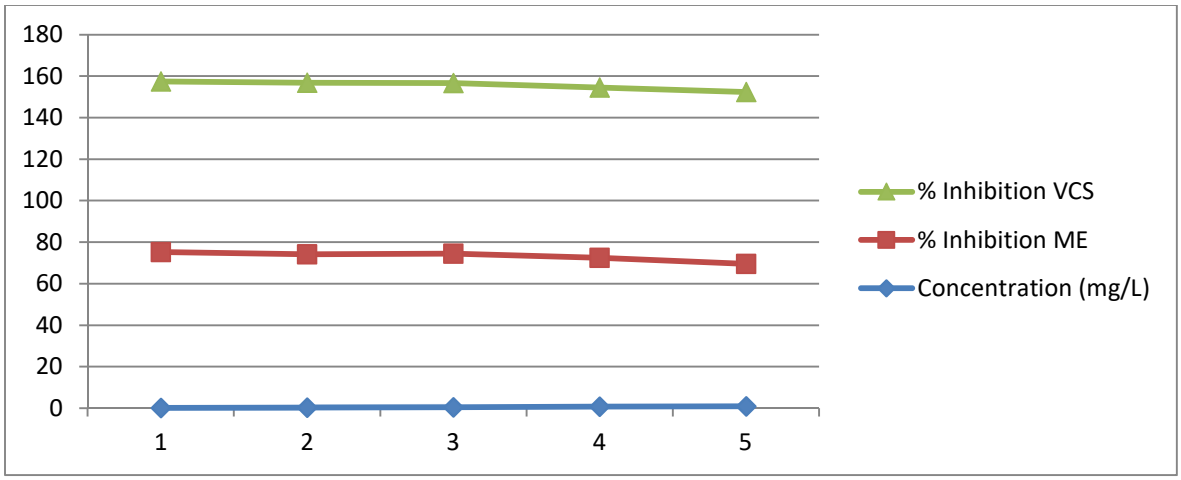

Figure 5: Plot of Concentration Vs % Inhibition for Methanol extract

Conclusion

This research showed that hexane, ethyl acetate, acetone and methanol extracts of leaf *Newbouldia laevis* may possess considerable antioxidant activities compared to vitamin C ascorbic acid (as positive controls).The DPPH radical scavenging assay shows that the extracts of methanol and acetone showed a good scavenging activity among all the extracts. The results obtained showed that this plant is very important from medicinal point of view and it needs further phytochemical exploitation to isolate phytochemical constituents showing antioxidant activity.

Declaration of conflicting interest

The authors declared no potential conflicts of interest.

References

[1] Radha, R.; Chauhan, P.; Puri, S.; Sharma, A.K.; Pundir, A (2001). **A study of wild medicinal plants used in Nargu Wildlife Sanctuary of district Mandi in Himachal Pradesh,** *India. J. Appl. Pharm. Sci.* 11, 135–144.

[2] Radha; Kumar, M.; Puri, S.; Pundir, A.; Bangar, S.P.; Changan, S.; Choudhary, P.; Parameswari, E.; Alhariri, A.; Samota, M.K.; *et al.*, (2021) **Evaluation of Nutritional, Phytochemical, and Mineral Composition of Selected Medicinal Plants for Therapeutic Uses from Cold Desert of Western Himalaya. Plants,** 10, 1429. https:// doi.org/10.3390

[3] Zengin, G, Cakmak YS, Guler GO, Aktumsek A (2011), *Rec Na. Prod,* **,5,** 123.

[4] Wang, L, Yen I, Liang H, Wu M (2003), *Journal of Food and Drug Analysis* 11, 60

[5] Veeru, P., Kishor, M. P. and Meenakshi, M (2009). **Screening of medicinal plant extracts for antioxidant activity**. *Journal of Medicinal Plants Research* 3(8), pp. 608-612,

[6] Rezaeian, S., Pourianfar, H. R. and Janpoor, J (2015). **Antioxidant properties of several medicinal plants growing wild in north eastern Iran.** *Asian Journal of Plant Science and Research*, 2015, 5(2):63-68

[7] Tsao A, Kim ES and Hong WK (2004), *Ca-Cancer Journal of Clinical* Medicine , 54, 150.

[8] Young, I.S and Woodside, J.V (2001). **Antioxidants in health and disease.** *Journal of Clinical Pathology*,54:176-186.

[9] Halliwell B (1994). **Free radicals, antioxidants, and human disease: curiosity, cause, or consequence?** *Lancet.* 344: 721-724.

[10] Ajibolu FC, Fashakin BJ, Fagbemi NT, Aluko RE (2011). **Effect of Pepti de size on antioxidant properties of African yam bean (*Sphenosty lisstenocarpa*) Protein Hydrosylate Functions.** *International Journal of Molecular Sciences* 12:6685- 6702.

[11] Oliver-Bever B (1986). **Medicinal Plants in Tropical West Africa.** Cambridge University Press, London. pp. 117-118, 16

[12] Burkill, H.M. (1997) **The useful plants of West Tropical Africa.** 2nd Edition, Vol. 4 (Families M-R), Royal Botanic Gardens, Kew.

[13] Iwu, M.M. (2000) **Handbook of African Medicinal Plants**. *CRC Press,* Inc., London, pp 19.

[14] Ogunlesi, M., Okiei, W., Ofor, E. and Awonuga, O. (2009) **Determination of the Concentration of Zinc and Vitamin C in Oysters and Some Medicinal Plants Used to Correct Male Factor Infertility.** *Journal of Natural Products,* 2, 89-97.

[15] Akinmoladun, A.C., Obutor, E.M. and Farombi, E.O. (2010). **Evaluation of Antioxidant and Free Radical Scavenging Capacities of Some Nigerian Indigenous Medicinal Plants.** *Journal of Medicinal Food,* 13, 444-45i.

[16] Amos, S., Binda, L., Vongtau, H., Chindo, B. and Abbah, J. (2002) **Sedative Effects of the Methanolic Leaf Extract of *Newbouldia laevis* in Mice and Rats.** *Bollettino Chimico Farmaceutico,* 141, 471-475.

[17] Ainooson, G.K., Woode, E., Obiri, D.D. and Koffour, G.A. (2009) **Antinociceptive Effects of (P. Beauv) Stem Bark Extract in a Rat Model. *Newbouldia laevis*** *Pharmacognos Magazine*, 5, 49-54.

[18] Hassan, S.W., Salawu, K., Ladan, M.J., Hassan, L.G., Umar, R.A. and Fatihu, M.Y. (2010). **Hepato-Protective, Antioxidant and Phytochemical Properties of Leaf Extracts of *Newbouldia laevis***. *International Journal of PharmTech Research*, 2, 573-584.

[19] Kuete, V., Wabo, H.K., Eyon, K.O., Feussi, M.T., Wiench, B., Krusche, B. and Effert, T. (2011). **Anticancer Activities of Six Selected Natural Compounds of Some Cameroonian Medicinal Plants.** PLoS ONE, 6,e21762.

[20] Bafor, E., Sanni, U. and Nworgu, Z.A. (2010) **In Vitro Determination of the Mechanism of the Uterine Stimulatory Effect *of Newbouldia laevis*.** *Pharmaceutical Biology,* 48, 808-815.

[21] Awemu, G.A., Okunrobo, L.O. and Awah, F.M. (2012) **Wound Healing and Antiulcer Activities of the Ethanol Extract of Newbouldia laevis Root Bark.** Journal of Pharmacy &Bioresources, 9, 29-33.

[22] Joppa, K.M., Vovor, A., Eklu-Gadegbeku, K., Agbonon, A., Aklikokou, K and Gbeassor, M.(2008). **Effect of Morindalucida Benth. (Rubiaceae) and Newbouldia leavis P. Beauv. (Bignoniaceae) on Sickling of Red Blood Cells.** *Medecine Tropicale: Revue du Corps de Sante* Colonial, 68, 251-256

[23] Usman H, Haruna AK, Akpulu IN, Ilyas M, Ahmadu AA, Musa YM. **Phytochemical and Antimicrobial Screenings of the Leaf Extracts of Celtisin tegrifolia *Lam***. *Journal of Tropical Biosciences.* 2005;5(2):72–76.

[24] Chang, L. C., and A. D. Kinghorn (2001). **Flavonoids as Cancer Chemopreventive Agents, In Tringali, Corrado (Ed.).**
Bioactive Compounds from Natural Sources.
London: Taylor and Francis. 159-185.

[25] Rahman, T.U., Khan, T., Khattak, K.F., Ali, A., Liaqat, W., and Zeb, M.A. (2016) **Antioxidant Activity of Selected Medicinal Plants of Pakistan.** BiochemPhysiol 5:208

[26] Sagar, B. K., and Singh, R. P. (2011). **Genesis and development of DPPH method of antioxidant assay.** *Food Sci Technol.* 48(4): 412–422.

Cite this article

Ushie, O. A., Egah, G.O.,Aasegh, T.J., Ibrahim, A.I., Bako, B., and Agyo, N.(2022).Antioxidant Activities of Hexane, Ethyl acetate, Acetone and Methanol Extracts of of*NewbouldiaLaevis*. FUAM Journal of Pure and Applied Science, **2**(**1**):76-81