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Comparative Analysis of Antioxidant Potential of The Fractions of Methanolic Root Bark Extract of *Annona senegalensis*

Idris Aliyu Kankara, Gayus Aminu Paulina*, Ibrahim Abdullahi
[1]Department of Science Laboratory Technology, Federal Polytechnic Kaura Namoda, Zamfara State-Nigeria

Abstract:- *Annona senegalensis* known as African custard apple or wild custard apple, Gwándàn dààjìì (Hausa) or dukuu-hi (Fulani) is a tropical perennial growing shrub (2-7m) or small tree (11m) growing under suitable environmental conditions. The present study aimed to compare the antioxidant potential of the fractions of methanolic root bark extract of *Annona senegalensis*. Methanolic root bark extract of *Annona senegalensis* was fractionated based on the preparative TLC. Silica gel G coated in preparative TLC plate was used as stationary phase with Chloroform: Methanol (9:1) as a mobile phase. The antioxidant potential of fractions was determined using DPPH assay. The result of preparative TLC revealed the presence of eleven bands labelled as fractions (I–XI) with different Rf value. Antioxidant activity assay of the fractions demonstrated a greater potential in the DPPH free radical scavenging activity. Fraction VIII (2.2267±0.02) has the highest antioxidant activity ($p<0.05$) compared to other fractions and to vitamin C standard (5.3980±0.01) while fraction I (6.2890±0.01) has the lowest antioxidant activity compared to vitamin C standard. The strong antioxidant activity possess by the root bark of *Annona senegalensis* could be beneficial in the treatment of ailments resulting from oxidative stress

Keywords:- *Annona senegalensis, DPPH and Preparative TLC.*

I. INTRODUCTION

Free radical are reactive oxygen species that are produced by body system in normal metabolism and during immune response to pathological cell metabolism, they are also triggered by exogenous chemical and several environmental factors such as smoke, (Saleh *et al.*,2010) and dust. Free radicals are capable of oxidizing biomolecules such as RNA, DNA, proteins and lipids resulting in lysis of cell membrane, cell death and tissue damage (Saleh *et al.*,2010). Ethnomedicinal plants have free radical scavenging activity, stimulate the activities of antioxidant enzymes and inhibit lipid peroxidation (Zhang and Zu, 2015). In an attempt to reduce the complication associated with the environmental pollution on humans, identification of medicinal plants with antioxidant potential has become a realistic and poweRful tool in ethnomedicinal community.

Annona senegalensis belong to the division of Magnoliophyta, class of Magnoliopsida, Subclass of Magnoliidae, order of Magnoliales, family of Annonaceae, Genus: *Annona* and the species of *senegalensi. Annona senegalensis* Pers. known as African custard apple or wild custard apple, Gwándàn dààjìì (Hausa) or dukuu-hi (Fulani) (Okoye *et al.*,2014; Mustapha, 2013) is a tropical perennial growing shrub (2-7m) or small tree (11m) under suitable environmental conditions. The bark is smooth to roughish, silver grey or grey-brown (Lall et al., 2017; Emmanuel *et al.*,2014). The leaves of this medicinal plant are ovate, alternate, simple, oblong, 6- 185 x 25- 120 mm in size, green to bluish green, mostly lacks hairs on upper suRface, with brownish hairs on lower suRface (Mustapha, 2013). Phytochemical screening of *Annona senegalensis* revealed the presence Alkaloids, Saponins (Konate *et al.*,2012),Tannins (Jada *et al.*,2014a), Resins, Reducing sugar, Carbohydrate (Okeye *et al.*,2010), Flavonoids (Jada *et al.*,2014b), Glycosides (Ijaiya, Arzika, & Abdulkadir, 2014), Phenol, Terpenoids (Konate *et al.*,2012; Okoye *et al.*,2012) steroids (Ijaiya *et al.*,2014) This plant is use as anti-snake venom (Molander *et al.*,2014; Chinyere *et al.*,2016; Adzu *et al.*,2005) male sexual impotence, erectile dysfunction (Nwonuma *et al.*,2015), anti-inflammatory (Adzu *et al.*,2003), anti-oxidant, antimicrobial (Apak and Olila, 2006) and antidiarrheal (Suleiman *et al.*,2008). The present study thus investigates and compared the antioxidant potential of the fractions of methanolic root bark extract of *Annona senegalensis.*

- *Sample Collection and Identification*

Fresh root bark of *Annona senegalensis* was randomly collected at Dan Nakwabo village in Kankara Local Government Area of Katsina State, Nigeria. The sample was transported to Biological science laboratory in specimen container. The sample was authenticated at the Herbarium section, Botany Unit, Bayero University Kano, Nigeria. The root bark of *Annona senegalensis* was thoroughly washed to remove sand and the drained parts were air dried.

- *Preparation of Plant Extract*

The root bark of *Annona senegalensis* was grounded using wooden mortar and pestle until powder was obtained to ensure homogeneity. Five hundred grams (500g) of shade dried powdered root bark of *Annona senegalensis* was immersed in methanol at a ratio of 1:5 (w/v) for 24 hrs with continuous stirring (at room temperature). The extraction was repeated for 3 days by changing the solvent every 24 hrs and the filtrate was concentrated using water bath. The dried extract was stored into labeled plastic containers prior to use.

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➢ *Fractionating of the Crude Extract*

The methanolic root bark extract of *Annona senegalensis* was fractionated based on the preparative TLC. Silica gel G coated in preparative TLC plate was used as stationary phase with Chloroform: Methanol (9:1) as a mobile phase. The solvent system was prepared in separate flask, 150ml was poured into the preparative TLC chamber. Thirty TLC plates (20cm x 20cm) were used for the thin layer chromatography (TLC) plates. Fifteen gram (15g) of the silica gel (adsorbent) was mixed with thirty ml of distilled water in a beaker and shaken vigorously for 90 seconds till slurry was formed. The plates were washed with distilled water and wiped, the wiped plates were mounted horizontally on a metal template and the slurry poured on the upper end of the central glass plate and spread evenly over the plates. The slurry was allowed to solidify on the plates, after solidification, the plates were activated in a scientific oven at 110^0C for 2hr (Rabel and Sherma, 2017; Udoidong *et al.*,2014). Excess adsorbent on the back and edges of the plates were wiped before developing the plates. The crude of methanolic root bark extract of *Annona senegalensis* was dissolved in chloroform and spotted to about 2 cm apart on a silica gel coated preparative TLC plate by using Pasteur pipepette and applied as a concentrated solution in a row of spots by three to four times on each plate; the spots was allowed to dried before the next application, 14 to 15 centimeter mark above the spot was placed. Then the plate was introduced into the TLC developing N- chamber (rectangular glass tank with inner dimensions of 21 cm×21 cm×9 cm) lined on all four sides with thick filter paper soaked with the mobile phase which allowed to stand for 1-2hrs to become saturated with the vapour phase. The mobile phase was allowed to reach the lower edge of the adsorbent, but the spot not immersed in the solvent. The cover was placed and the system was maintained until the mobile phase ascending to point 14 cm above the baseline. Then, the TLC plate was removed and viewed under the ultra violet (UV) light at 254nm and 345nm (Hamid and Kadhim, 2016). Several bands were detected from the plates, their color and retention factor were noted. The bands were scraped out with a razor blade and dissolved in methanol; the mixture was allowed to stand for thirty minutes. The mixture was filtered, and the filtrates were stored in a clean container for further analysis.

➢ *DPPH Radical Scavenging Assay*

Fraction of methanolic root bark extract of *Annona senegalensis* at different concentrations (2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml) were mixed with 3ml of methanolic solution of DPPH (0.1mM). After an incubation period of 30 minutes in the dark at ambient temperature, the absorbance was read at 517 nm wavelength. The inhibition of free radical DPPH by Ascorbic acid, reference antioxidant compound was also analyzed with the same concentrations and the same conditions for comparison.

The inhibition of free radical DPPH percentage (I %) was calculated as follows

$$\%\ INHIBITION = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Where Absorbance of control is the absorbance of the DPPH and Absorbance of sample is the absorbance of the test compound (containing all reagents and the test product). A graph of % Inhibition against the various concentrations was plotted and IC_{50} values were extrapolated from the graph. The value IC_{50} is the concentration of the fractions which reduces the initial DPPH concentration by 50% it is used to characterize the antioxidant activity of the fractions of root bark of *Annona senegalensis*. All tests were performed in triplicate for each concentration (Alilou *et al.*,2014; Chen *et al.*,2004)

➢ *Statistical Analysis*

Data were expressed as the mean ± SD of three determinations. Mean was analyzed between the groups using a one-way ANOVA and Duncan multiple range test with SPSS 20.0 version. Differences were considered statistically significant at $P < 0.05$.

II. RESULT

Fractionating of crude methanolic root bark extract of *Annona senegalensis* by preparative thin layer chromatography plates were carried out on the chloroform: methanol (9:1). The results are presented in (Figure 1), eleven bands were detected labeled as fractions (I–XI) and the Rf value of each fraction was recorded in (Table 1).

Fraction	Solute front	Rf values
Fraction I	12.3	0.93
Fraction II	11.5	0.88
Fraction III	11	0.84
Fraction IV	10	0.76
Fraction V	9	0.69
Fraction VI	8.7	0.66
Fraction VII	6.7	0.51
Fraction VIII	6	0.46
Fraction IX	4.5	0.34
Fraction X	3.5	0.27
Fraction XI	1.9	0.15

Table 1:- Results of preparative TLC, using chloroform: methanol (9:1) as solvent system which 13.1cm as solvent front

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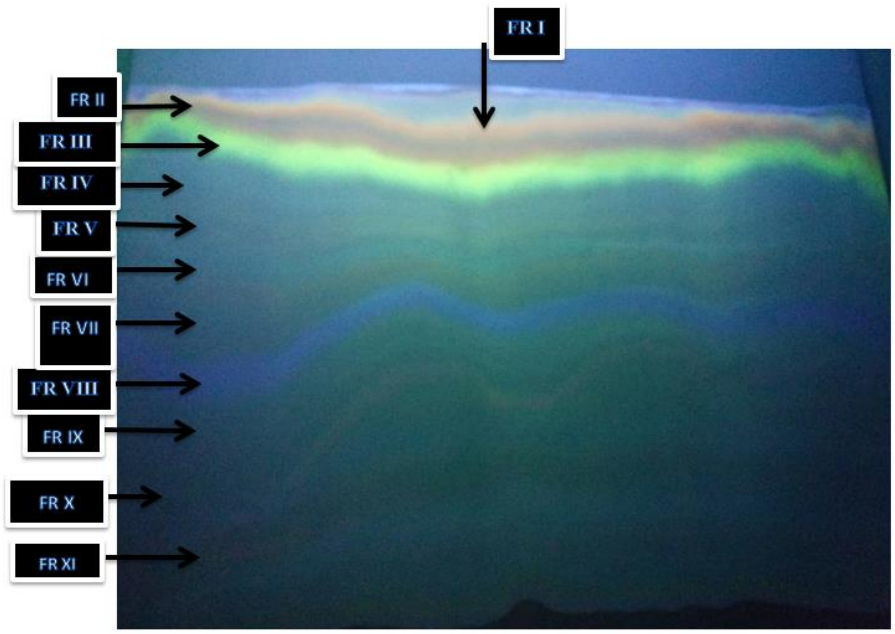

Fig 1:- Preparative TLC chromatogram showing different bands; fractions (FR I–FR XI) of the methanolic extract root bark *Annona senegalensis*

The result of DPPH radical scavenging activity of fractions of methanolic root bark extract of *Annona senegalensis* was presented in (Table 2) which were expressed as IC50. And the dose-response curve of DPPH radical scavenging activity of fractions (I-XI) of methanolic root bark extract of *Annona senegalensis* and vitamin C were shown in figure 2 and 3. Fraction VIII (2.2267±0.02), Fraction VI (2.5027±0.04), Fraction III (2.5700±0.06) and Fraction X (2.9217±0.01) have the highest antioxidant activity ($p<0.05$) followed by fraction IX (4.0980±0.01), fraction V (4.6963±0.03) and fraction VII (4.9283± 0.01) compared to the activity of vitamin C (5.3980±0.01) standard. While fraction XI (5.5380±0.01) and fraction II (6.2500±0.01) have the low antioxidant activity, but fraction I (6.2890±0.01) was found to have the lowest activity ($p<0.05$) compared to the antioxidant activity of vitamin C standard.

Fractions of *Methanolic root bark extract of Annona senegalensis*	IC_{50} Value (µg/ml)
Vit. C	5.3980±0.01
FR I	6.2890±0.01*
FR II	6.2500±0.01*
FR III	2.5700±0.06**
FR IV	5.3967±0.03*
FR V	4.6963±0.03*
FR VI	2.5027±0.04**
FR VII	4.9283± 0.01*
FR VIII	2.2267±0.02**
FR IX	4.0980±0.01*
FR X	2.9217±0.01**
FR XI	5.5380±0.01*

Table 2:- DPPH radical scavenging activity of fractions of methanolic extract root bark of *Annona senegalensis*

*** Significant difference between fractions and vitamin C standard < 0.05. **Highly significant difference between fractions and vitamin C standard p < 0.05. Data showed as mean ± SD (n = 3) per group**

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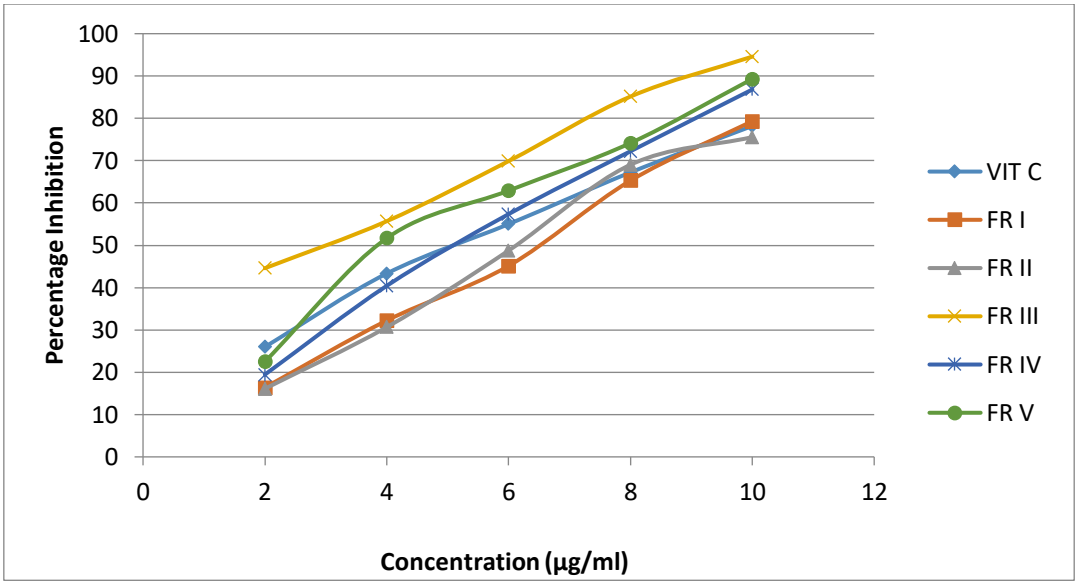

Fig 2:- DPPH scavenging activity of fractions (VI-XI) of methanolic extract root bark of *Annona senegalensis*

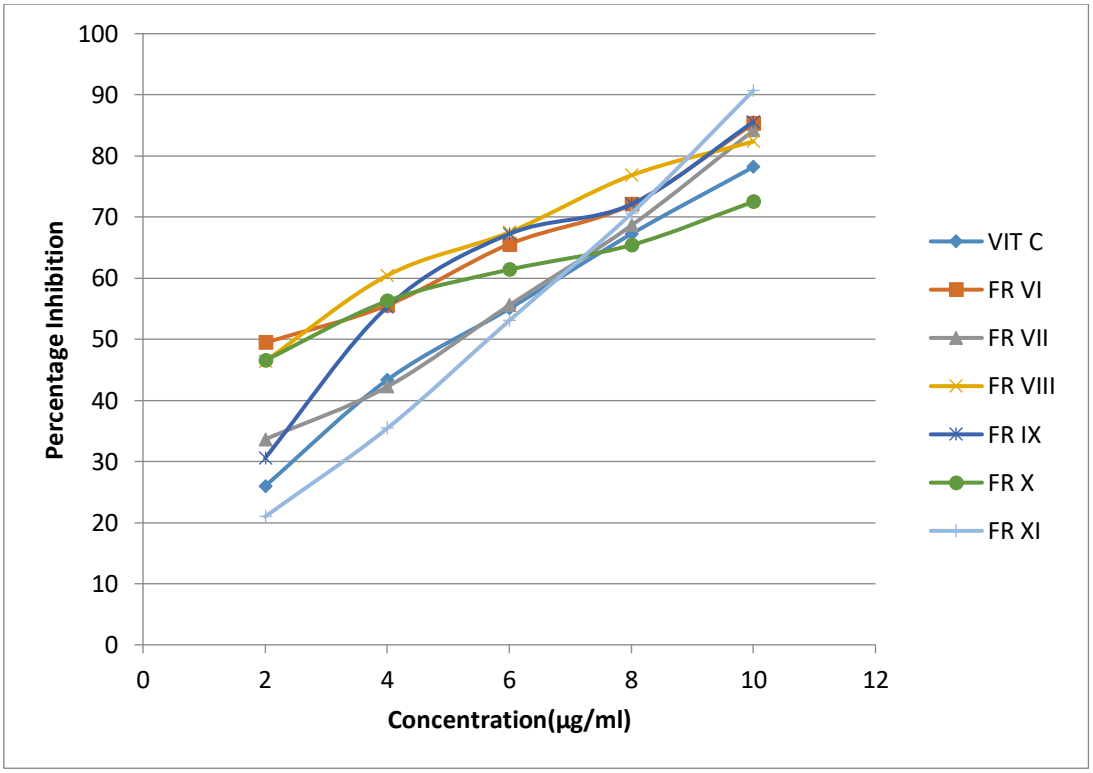

Fig 3:- DPPH scavenging activity of fractions (VI-XI) of methanolic extract root bark of *Annona senegalensis*

III. DISCUSSION

Preparative thin-layer chromatography is a valuable liquid chromatographic method for isolating of compounds, in amounts of 10-1000 mg for biological activity assay, analytical purposes and for structural elucidation. Preparative TLC profile of the crude methanolic root bark extract of *Annona senegalensis* gave an impressive result that indicated the presence of eleven fractions (Fig. 1) with different Rf values (Table 1) reflecting the presence of diverse type of phytochemicals in this plant. This variation in Rf values of the fractions provides a very valuable information in understanding of their polarity. Fractions (I-VII) have the highest Rf value in less polar solvent system (Chloroform: methanol; 9:1 v/v) used, this indicated that the phytochemicals present in those fractions might have low polarity while fractions (VIII-XI) have lower Rf value indicating that those fractions might have phytochemicals with high polarity. When the antioxidant potential of fractions (I-XI) was tested using DPPH free radical scavenging assay, the fractions reduced the DPPH solution with the changed of deep violet color to pale yellow color. Fraction III, XI and VIII have lower IC_{50} values, demonstrated a greater potential in the DPPH free radical scavenging test (Brand-Williams *et al.*,1995). The antioxidant activity of fraction VIII, IV and III were

approximately two times higher than the activity of vitamin c and three times than other fractions tested in this study. Fraction VIII showed highest antioxidant potential compared to other fractions as well as ascorbic acid. This indicated that *Annona senegalensis* root bark could serve as good source antioxidants since IC_{50} value obtained in this study is <50µg/ml (Ervina *et al.*,2016). The results obtained in this study from all the fractions analysed were classified as very powerful antioxidants as compare to vitamin C, this result was in line with the finding of Yande *et al.* (2017). The DPPH radical scavenging activity of fractions of methanolic root bark of *Annona senegalensis* could be due to the ability of the phytochemicals present in the fractions to denote an H^+ to DPPH solution (Balasundram *et al.*,2005) thereby reducing the DPPH solution. The DPPH molecule is similar to free radicals formed in the body system during normal cell metabolism, immune response to pathological cell metabolism and the activity of these free radicals could be suppressed by the fractions of methanolic root bark of *Annona senegalensis*, probably due to the ability of the phytochemicals present in the fractions to activate the activity of the antioxidant enzymes (Zhang and Zu, 2015; Housam, Warid, and Zaid, 2014). Yande *et al.* (2017) in their work attributed the strong antioxidant activity of the extract of *Annona senegalensis* to the large amount of total phenols in the plant. The lower IC_{50} value obtained in the present study, indicated that apart from total phenols, *Annona senegalensis* might have other phytochemical compound(s) that could act as antioxidant agent(s).

REFERENCES

[1]. Adzu, B., Abubakar, M., Izebe, K., Akumka, D., and Gamaniel, K. (2005). Effect of Annona senegalensis root bark extracts on Naja nigricotlis venom in rats. *J Ethnopharmacol, 96*, 507-513.

[2]. Adzu, B., Amos, S., Adamu, M., and Gamaniel, K. (2003). Anti-nociceptive and anti-inflammatory effects of the methanol extract of Annona senegalensis root bark. *J Nat Rem, 3*, 63-67.

[3]. Alilou, H., Asdadi, A., Hassani, L., González-Mas, M., Blázquez, M. A., and Akssira, M. (2014). Antifungal and Antioxidant Activity of Asteriscus graveolens subsp. odorus Essential Oil. *Journal of Natural Sciences Research, 4*(10).

[4]. Apak, L., and Olila, D. (2006). The in vitro antibacterial activity of Annona senegalensis, Securidacca longipendiculata and Steganotaenia-Ugandan medicinal plants. *Afr Health Sci*, 31-35.

[5]. Atawodi, S., Ameh, D., Ibrahim, S., Andrew, J., Nzelibe, H., and Onyike, E. (2003). Indigenous knowledge system for treatment of typanasomiasis in Kaduna State of Nigeria. . *J Ethnopharmacol*, 279-282.

[6]. Balasundram, N., Yew Ai, T., Sambanthamurthi, R., Sundram, R., and Samman, S. (2005). Antioxidant properties of palm fruit extracts. *Asia Pac J Clin Nutr 2005; 4 (4):319-324, 4*, 319-324.

[7]. Brand-Williams, W., Cuvelier, M., and Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *Lebensm-Wiss u-Technol, 28*, 25-30.

[8]. Chen, C., Weng, M., Wu, C., and Lin, J. (2004). Comparison of radical scavenging activity, cytotoxic effects and apoptosis induction in human melanoma cells by taiwanese propolis from different sources. *Evidence Based Complementary Alternative Medicine, 1*, 175-185.

[9]. Emmanuel, A., Ebinbin, a., and Amlabu, w. (2014). Detoxification of Echis ocellatus venom-induced toxicity by Annona senegalensis Pers. *Complement Integr Med.*, 93–97.

[10]. Ervina, M., Nawu, Y., and Esar, S. (2016). Comparison of in vitro antioxidant activity of infusion, extract and fractions of Indonesian Cinnamon (Cinnamomum burmannii) bark. *International Food Research Journal, 23*(3), 1346-1350.

[11]. Ezugwu, C., and Odoh, U. E. (2003). Anticonvulsant activity of the root extract of Annona senegalensis. *J Trop Med Plants, 4*(1), 51-55.

[12]. Hamid, H. K., and Kadhim, E. (2016). Extraction, isolation and characterization of Pyrrolizidine Alkaloids present in Senecio vulgaris Linn grown in Iraq. *Journal of Pharmacognosy and Phytochemistry , 5*(6), 28-37.

[13]. Housam, H., Warid, K., and Zaid, A. (2014). Estimating The Antioxidant Activity For Natural Antioxidants (Tocochromanol) and Synthetic one by DPPH. *International Journal of Pharmacy and Pharmaceutical Sciences, 5*(6), 441-444.

[14]. Ijaiya, I., Arzika, S., and Abdulkadir, M. (2014). Extraction and Phytochemical Screening of the Root and Leave of Annona senegalesis (Wild Custad Apple). Aca. *Journal of Interdisc Stu., 3*(7), 9-15.

[15]. Jada, M., Usman, W., and Adamu, Y. (2014). In vitro Antimicrobial Effect of Crude Tannins Isolated from the Leaf of Annona senegalensis. *Int. J Biochem Res Rev, 4*(6), 615-623.

[16]. Jada, M., Usman, W., and Olabisi, A. (2015). Crude Flavonoids Isolated from the Stem Bark of Annona senegalensis have Antimicrobial Activity. *J of Adv Bio Biotech, 2*(1), 24-29.

[17]. Konate, A., Sawadogo, W., Dubruc, F., Caillard, O., Ouedraogo, M., and Guissou, I. (2012). Phytochemical and Anticonvulsant Properties of Annona senegalensis Pers. (Annonaceae),Plant Used in Burkina Folk Medicine to Treat Epilepsy and Convulsions. *British Journal of Pharmacology and Toxicology, 3*(5), 245-250.

[18]. Lall, N., Kishorea, N., Bodibaa, D., Morea, G., Tshikalangea, E., Kikuchia, H., and Oshima, Y. (2017). Alkaloids from aerial parts of Annona senegalensis against Streptococcus mutans. *Natural Product Research*, 1944–1947.

[19]. Mpiana, P. (2012). Antisickling properties, thermal and photochemical degradations of anthocyanin extracts from Annona senegalensis (Annonaceae). *Int. J Biol Chem Sci. 2012, 6*(5), 2241-2251.

[20]. Mustapha, A. A. (2013). Annona senegalensis Persoon: A Multipurpose shrub, its Phytotherapic, Phytopharmacological and Phytomedicinal Uses. *International Journal of Science and Technology*, 862-865.
[21]. Ngamo, T. L., Goudoum, A., Ngassoum, .., M. L., Malaisse, F., and Hance, T. (2007). Chronic Toxicity of Essential Oils of 3 Local Aromatic Plants towards Sitophilus zeamais Motsch. (Coleoptera:Curculionidae). *Afr. J Agric Res*, *2*(4), 164-167.
[22]. Ngbolua, K., Moke, E., Baya, J., Djoza, R., Ashande, C., and Mpiana, P. (2017). A mini-review on the pharmacognosy and phytochemistry of a tropical medicinal plant: Annona senegalensis Pers. (Annonaceae). *Tropical plant research*, 168–175.
[23]. Nwonuma, C., Irokanulo, E., Jolaiya, A., and Ore, A. (2015). Effect of Aqueous Leaf Extract of Annona senegalensis on Selected Testicular Function Indices of Wistar Rats. *American Journal of Life Sciences*, *3*(3), 203–212.
[24]. Odoh UE, E. C. (2004). Preliminary investigation of the analgesic activity of the chloroform root extract of Annona. *senegalensis L. J Pharm Allied Sci 2004*, *2*(1), 169-172.
[25]. Okoye, T., Akah, P., Nworu, C., and Ezike, A. (2014). Kaurenoic Acid Isolated from the Root Bark of Annona senegalensis Induces Cytotoxic and Antiproliferative Effects against PANC-1 and HeLa cells. *European Journal of Medicinal Plants*, 579-589.
[26]. Onwusonye, J. C., Uwakwe, A. A., Iwuanyanwu, P., and Iheagwam, U. (2014, July). Oral acute toxicity (LD50) study of methanol extract of Annona senegalensis leaf in albino mice. *Sky Journal of Biochemistry Research*, *5*, 46-48.
[27]. Rabel, F., and Sherma, J. (2017). Review of the State of the Art of Preparative Thin Layer Chromatography. *Journal of Liquid Chromatography and Related Technologies*, 1-48.
[28]. Saleh, M. A., Clark, S., Woodard, B., and Deolu-Sobogun, S. (2010). ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITIES OF ESSENTIAL OILS. *Ethnicity and Disease*, *20*, S1-78–S1-82.
[29]. Suleiman, M., Dzenda, T., and Sani, C. (2008). Antidiarrhoel activity of the methanol stem-bark extract of Annona senegalensis Pers. *J Ethnopharmacol 2008*, *16*(1), 125-130.
[30]. Udoidong, A. A., Etuk, B. A., and Udo, I. E. (2014). Phytochemical and chromatographic analysis of chloroform extract of Marsdenia latifelia. *Advances in Applied Science Research*, *5*(1), 53-58.
[31]. Yande, K. H., Toghueo, K., J.B., F. H., Souleymanou, A., Ngouana, V., and Boyom, F. (2017). Phenol content and Antioxydant activity of crude extracts and fractions from Annona senegalensis. *Journal of Medicinal Plants Studies 2017*, *5*(5), 289-294.
[32]. Zhang, L., and Zu, J. (2015). Comparative study on antioxidant activity of essential oil from white and black pepper. *European Journal of Food Science and Technology*, *3*(3), 10-16.