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Research Article

Assessment of *In-vitro* Antioxidant Activities and Genotoxicity in *E. coli* of Ethanol Extracts of *Vitellaria paradoxa* (Gaertn. F)

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Abstract
Medicinal plants grossly contain bioactive phytochemicals. Phytochemical such as polyphenols, flavonoids, saponins, vitamin C, E have antioxidant potentials; they therefore act as protecting agent when used in the right perspective. The objective of this study is to evaluate the *in vitro* antioxidant and genotoxic properties of ethanol extracts of *Vitellaria paradoxa*. To achieve this, different antioxidant assays were used whereas SOS chromotest was used for the assessment of genotoxicity. Using ascorbic acid as a standard, the leaf extract had a 1.4 fold higher level total antioxidant when compared to seed. At 800 µg/ml, the leaf extract compared well to the standard in reducing power but that of the seed extract was significantly lower. The % inhibition of 1,1-diphenyl-2-picrylhydrazyl (DPPH) for leaf and seed extracts at 50 µg/ml are 79.95 ± 0.31 and 82.52 ± 0.31 respectively which are significantly low compared to value by ascorbic acid (93.04 ± 2.07). The phytochemical analysis showed the total phenolic content was (83.46 ± 0.72 and 68.88 ± 7.50) µgGAE/ml for leaf and seed extracts respectively. The leaf extract total flavonoid content was equally significantly higher in comparison to the seed using quercetin as a standard compound. The genotoxic assay, SOS chromotest, showed both extracts to be non-genotoxic at concentrations < 800 µg/ml in comparison to 4-Nitroquinoline-1- oxide (4-NQO). However at 800 µg/ml the extracts showed marginal genotoxicity. Findings from this study showed both extracts of *Vitellaria paradoxa* have antioxidant properties. The leaf extract showed a more promising antioxidant potential and high level of flavonoid. The marginal genotoxicity observed at 800 µg/ml calls for caution on the medicinal application of the plant.
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Key Words: Antioxidant, genotoxicity, medicinal plants, *Vitellaria paradoxa*

INTRODUCTION

Plants are the primary producers for most animals. Humans as the highest order of animals use plants for various purposes such as food, clothing, furniture’s, building, medicines and so on. Medicinal plants have been defined as those plants with one or more parts containing bioactive chemical substances which can be used in preventive, protective and therapeutic purposes against ailment or which have precursors for the synthesis of useful drugs (Kumar *et al*., 2010). In developing nations, about 80% of the population relies on medicinal plants as their first source of primary health care (Akinyemi, 2000; Abushama *et al*., 2014; Vasanthi *et al*., 2014; Fodouop *et al*., 2017).

It is now agreed that that bioactive components of medicinal plant reside in the secondary metabolites commonly called the phytochemicals (Olorunnisola *et al*., 2012; Seladji *et al*., 2014; Prasad *et al*., 2014; Ehimwema and Osarieme, 2014). These bioactive components are polyphenols such as flavonoids, tannins, phenolics, saponins, alkaloids (Muanda *et al*., 2011; Amari *et al*., 2014; Olugbami *et al*., 2015). In addition, to polyphenols we have some vitamins (C, E), carotenoids and some others acting as antioxidant component in several plants. These antioxidants act as scavengers of free radicals, inhibitors of peroxidation and chelators of transition metals (Amari *et al*., 2014; Ehimwenma and Osarieme, 2014). Therefore, they help in protecting and preventing a lot of diseases such as diabetes, Parkinson's, inflammation, Alzheimer's , atherosclerosis, stroke, cancer e.t.c (ôzgen *et al*., 2006, Tepe *et al*., 2005, Olugbami *et al* ., 2015; Abolaji *et al*., 2018).

It is worthy to note that most traditional medicinal plant have never been the subject of exhaustive toxicological test as it is required for pharmaceutical compounds. Based on their traditional use for a long period of time, they are assumed to be safe. However, studies have shown a lot of plants used as food ingredients or in traditional medicine to be toxic, mutagenic and/or carcinogenic (Cardoso *et al*., 2006; Mohd Fuat *et al*., 2007). The short term SOS chromo test assay which makes use of *Escherichia coli* PQ37 bacterial cell is a tool for assessment of toxicity of such plants extracts. It is useful for the assessment of the presence of primary DNA damaging agent. In addition, it is highly sensitive and dependable (Chaabane *et al*., 2012; Kocak, 2015)

One promising indigenous Africa plant is *Vitellaria paradoxa* (Gaertn. F), a member of the Sapotacea family. It is commonly called shea butter tree as the source of shea butter cream produced from the seed (Wei *et al*., 2019). The local names in the three major language of Nigeria are Emi (Yoruba), Okwuma (Igbo) and Kadaya (Hausa) (Hall *et al*., 1996). Different parts of the plant: root, stem bark, leaf, seed are traditionally used in treatments of various enteric infection such as diarrhea, dysentery, skin diseases, wounds, helmethes and gastro intestinal infection (Saladoye *et al*., 1982). In addition root and root bark are ground to paste and taken orally to cure jaundice (Ampofu, 1983). Earlier researches have

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reported it to have protective effect against acetaminophen-induced hepatotoxicity (Ojo *et al*., 2006), to be antidiabetic (Coulibaly *et al.,* 2014), anti-inflammatic / anti-arthritic (Foyet *et al*., 2015), to ameliorate scopolamine-induced memory impairment (Foyet *et al.*,2016) to have antibacterial properties (Fodouop *et al.,* 2017) and showed antiproliferative ability (Tagne *et al*., 2014). However, there is no information as to whether the above medicinal properties relate to the antioxidants properties of the extracts of *Vitellaria paradoxa*. In addition, there is dearth of information on its toxicities. Hence the objective of this study is to determine the *in vitro* antioxidant activity and genotoxic potential in *E. coli* of ethanol extracts of *Vitellaria paradoxa* leaves and seeds

MATERIALS AND METHODS

Chemicals: The main reagents used in this study are ammomium molybdate, ferric chloride, 2,2-diphenyl-1-picryl hydrazyl (DPPH), Folin-ciocalteus phenol reagent, Aluminum chloride, potassium ferricyanide, ascorbic acid, gallic acid, quercetin, ethanol. These and other reagents were of analytical grade and products of Sigma Chemical Co. (St. Louis, USA) or BDH Chemical Ltd, Poole, UK.

Bacterial strain and culture: The SOS chromotest kit was purchased from Environmental Bio-detection Product Inc (ebpi). The bacterial strain used (*E. coli* PQ37) is a component of the kit. The content of the growth media was transferred into the vial containing the bacterial cell and was given a quick shake to ensure it is well mixed. It was incubated overnight for 16 hours at 37 ℃ until an optical density of 0.3 at 600nm according to the manual instructions.

Plant sample collections: Fresh leaves and seed of *V.paradoxa* were collected from Saki, Oyo State, Nigeria between March – June, 2016 at intervals and was identified and authenticated at the herbarium of the Department of Botany, University of Ibadan, Nigeria. The voucher specimen of the plant was deposited in the herbarium of same department with the voucher No: UIH-22624.

Preparation of leaf extract: The fresh plant leaves were washed to remove all sand and air-dried at room temperature in the Department of Biochemistry, University of Ibadan. Thereafter they were milled to powder using a mechanistic blender. The powdery samples were used for the extraction.

Preparation of seed extract: The Kernel were washed and air-dried. They were then broken to get the seed which was cut into bits and oven-dried at 60℃ for 2hrs. It was milled and defatted using petroleum ether in a soxhlet extractor. The resultant cake was extracted as seed extract.

Ethanol extraction of leaves and seeds: The milled leaf sample or defatted cake of the seed respectively was extracted in ethanol at room temperature. Exactly 500g of the powdery leaf and seed were emacerated in 2500 ml of 70% ethanol for 72 hours at room temperature with constant stirring and shaking at intervals. The extracts were filtered and the filtrates concentrated using a rotary evaporator at temperature of 40 ℃. The extracts was kept in the refrigerator at 4 ℃ until when needed

In vitro antioxidant activity assays

Total antioxidant capacity assay using phosphomolybdenum method: The total antioxidant was determined as described by Pieto *et al*., 1999. The principle is based on the reduction of phosphomolybdenum Mo (vi) to Mo (v) which leads to the formation of a green complex at acidic pH. Using ascorbic acid as standard an aliquot of 0.3 mL (300 µL) of extracts solution was mixed with 3 mL of reagent mixture (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Thereafter the tubes were capped and incubated in water bath at 95 ℃ for 90 minutes after which they were allowed to cool. Absorbance for each sample was read at 696 nm using UV/VIS spectrometer 725S. The antioxidant capacity is reported as µg of ascorbic acid equivalent (AAE) per mL.

Reducing power assay: The reducing power ability of the extracts were assayed for by the transformation of Fe^{3+} - Fe^{2+} in the presence of the extracts as described by Oyaizu, (1986). 1ml of varying concentrations of extracts (25-800 µg/ml), 2.5ml of phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide was incubated at 50℃ for 10 minutes. 2.5ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000rpm for 10 minutes. About 2.5 ml of the supernatant was diluted with 2.5 ml of distilled water and mixed with 0.5 ml of freshly prepared 0.1% ferric chloride. The absorbance was read at 700nm. Ascorbic acid was used as the standard and all tests were performed in triplicate.

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay: The radical scavenging activity of the extracts was determined according to the method of Brand-williams *et al*., (1995). The stable DPPH radical can easily accept a hydrogen radical donated hence leading to the loss of violet/ purple colour. This is characterised by an absorption in ethanol solution centered at about 517nm. Briefly 1 ml of 0.5 Mm DPPH solution was mixed with 1 ml of varying concentrations (50 – 1000 µg/ml in ethanol) of each extract or standard (ascorbic acid). The mixture was kept in the dark for 30 minutes at room temperature and the absorbance read at 517nm using UV/VIS spectrophotometer 752S. The radical scavenging activity was calculated using the following equation.

DPPH radical scavenging activity (%) = $(A_0 - A_1)/A_0 \times 100$

Where A_0 is the absorbance of control and A_1 the absorbance of standard or extract samples.

Total phenol content determination: The total phenol in the extracts were determined according to the method of Kim *et al* ., (2003). It is based on the basic principle that a phenol loses a H^+ ion to produce a phenolate ion which reduces folin-ciocalteu reagent. 0.5 ml of each extract sample (800µg/ml) was added 1 ml of 1:10 dilution of folin-ciocalteau reagent. After 5 minute, 10 ml of Na_2CO_3 (7%) solution was added to the mixture followed by the addition of 13 ml of distilled water, vortexed and kept in the dark for 90 minute at 25℃ . The absorbance was read at 750 nm. All tests were performed in triplicate and graph was plotted with the mean values of the three determinations. Results are expressed as microgram of

gallic acid equivalent per milligram of dry weight (µg GAE/mg) of extract.

Estimation of total flavonoid content: Total flavonoid content (TFC) were determined as described by Zhisten *et al*., (1991). 0.5 ml of 2% ethanol aluminum chloride ($AlCl_3$) solution was mixed with 0.5 ml of each extract (800 µg/ml) and allowed to stand for 45 minute at room temperature. The absorbance was read thereafter at 420 nm. Quercetin was used as the reference standard. All samples were performed in triplicate and the TFC values expressed as microgram of quercetin equivalent per milligram (µg QE/mg) of extracts.

Genotoxicity assay: The SOS chromotest with *E. coli* PQ37 strain was performed according to Quillardet et al., (1982). Briefly the bacterial cell gotten from the exponential phase culture was diluted 1: 9 into fresh medium. 10 µl of diluent (10% DMSO) was placed in the wells of the microplate with the exception of column A. Thereafter 20 µl of 4-Nitroquinoline-1- oxide (4-NQO) and the test extracts was placed in column A's, six series of two fold dilutions was subsequently done. 100 µl of the prepared bacterial was added to each well containing either 4-NQO or extracts {ethanol leaf extract of Vitellaria paradoxa (ELVp) , ethanol seed extract of Vitellaria paradoxa (ESVp)}. The plate was incubated for 2 hours at 37°C. Using the simultaneous activity check of β-galacctosidase and alkaline phosphatase, 100 µl of a chromogenic substrate (gotten from the mixture of the blue chromogen and dry alkaline phosphate substrate) was added into each well. It was there after incubated for 90 minutes at 37°C until a green colour appeared. 50 µl of stop solution was added to each wells. Absorbance was read using a microplate reader at 600 nm to determine β-galacctosidase production (genotoxicity) and 420 nm to determine viability of bacterial against negative control. The SOS inducing factor (SOSIF) was calculated as shown below.

SOSIF =
{(OD600i)/(OD420i)}/{(OD600, negative)/(OD420, negative)}

Where OD600i and OD420i are the absorbances of sample (control or extracts) at a specific concentration at each indicated wavelength, and OD600 negative and OD420 negative are the absorbance of the negative control at the indicated wavelengths (600 and 420 nm).

RESULTS

The percentage (%) yield of both samples was calculated after extraction of the leaf and seed samples of *Vitellaria paradoxa*. The percentage yield in for ethanol leaf extract of *Vitellaria paradoxa* (ELVp) was 28.53 ± 2.17 % while for ethanol seed extract of *Vitellaria paradoxa* (ESVp) was 23.99 ± 2.03 % as shown in figure 1 below. The total antioxidant capacity of the extracts was in comparison with the standard compound ascorbic acid. From the standard curve of the ascorbic acid as shown in figure 2 below the equation was y = 0.0024X - 0.027 with a R^2 value of 0.9875. The total antioxidant capacity of ELVp extract is 362.6 ± 31.76 µgAAE/mL and that of ESVp extract as 237.8 ± 4.174 µgAAE/mL as presented in figure 3. The figure showed a significant decrease (p<0.005) of the total antioxidant capacity of the ethanol seed of *Vitellaria paradoxa* (ESVp) when compared with the ethanol leaf extract of *Vitellaria paradoxa* (ELVp).

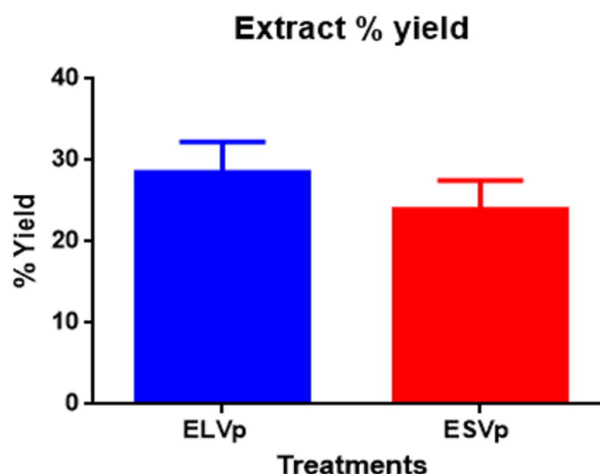

Figure 1:
Percentage yield of leaf and seed extract of *Vitellaria paradoxa*. ELVp represents the ethanol leaf extract of *Vitellaria paradoxa* while ESVp represents the ethanol seed extract of *Vitellaria paradoxa*

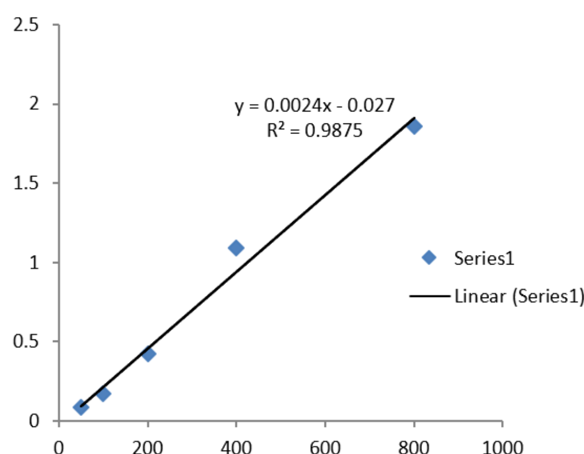

Figure 2:
Standard curve of total antioxidant capacity using ascorbic acid.

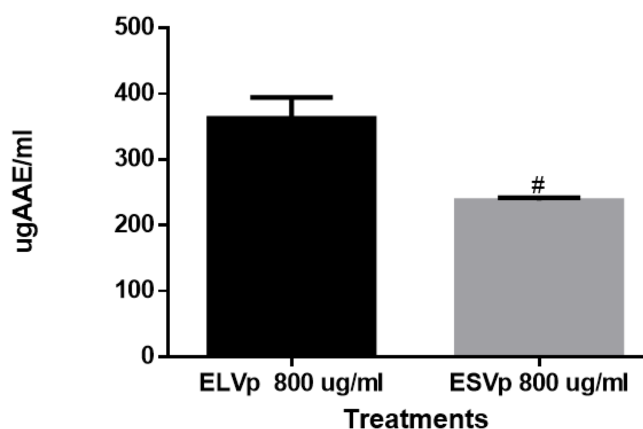

Figure 3:
The total antioxidant capacity of *V.paradoxa* extracts
significant (P<0.005) when compared to ethanol leaf extract of *Vitellaria paradoxa* (ELVp) treatment. ELVp represents the ethanol leaf extract of *Vitellaria paradoxa* while ESVp represents the ethanol seed extract of *Vitellaria paradoxa*.

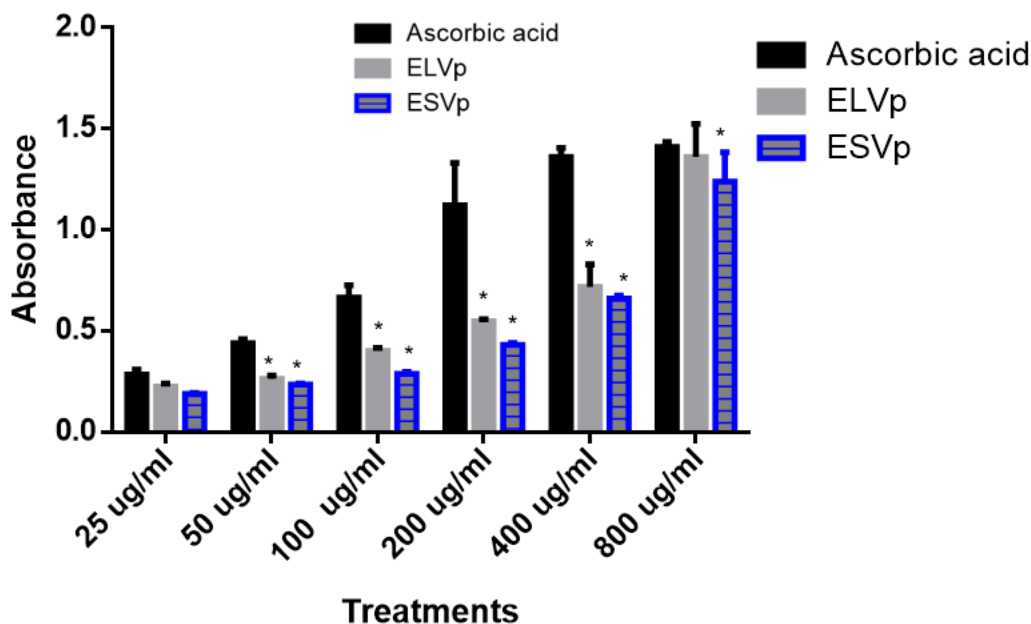

Figure 4:
Reducing power scavenging activity of V. *paradoxa* extracts and positive control (ascorbic acid). * represents significant when compared to control (ascorbic acid). ELVp represents the ethanol leaf extract of *Vitellaria paradoxa* while ESVp represents the ethanol seed extract of *Vitellaria paradoxa*

The result for reducing power scavenging activity of ascorbic acid and *Vitellaria paradoxa* leaf and seed extracts showed a concentration dependent scavenging ability. When compared to the standard (ascorbic acid) both extracts was significantly lower at concentration 50 µg/ml to 400 µg/ml. However at 800 µg/ml there was no significant difference between the leaf extract (ELVp) and the standard (ascorbic acid) as shown in figure 4 below.

Figure 5 shows the DPPH scavenging ability of the extracts. The result showed both extract have a lower percentage inhibition for DPPH when compared with ascorbic acid.

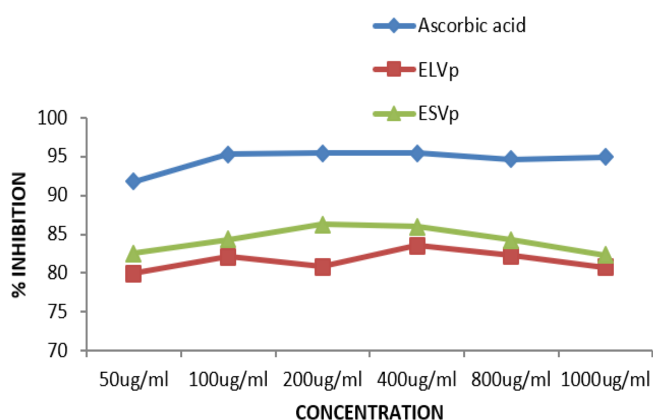

Figure 5:
DPPH radical scavenging activity of *V.paradoxa* extracts and positive control (ascorbic acid). ELVp represents the ethanol leaf extract of *Vitellaria paradoxa* while ESVp represents the ethanol seed extract of *Vitellaria paradoxa*.

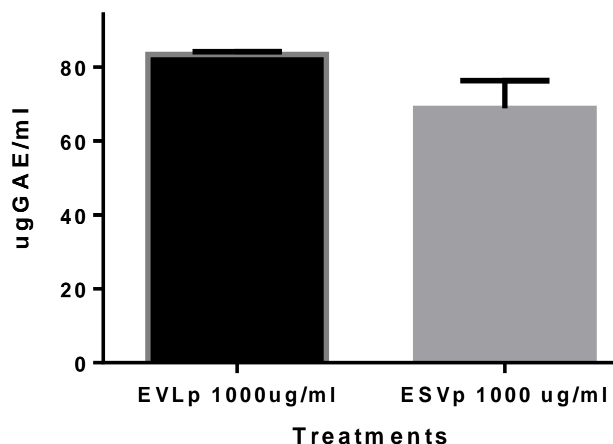

Figure 6:
Total phenol content of *Vitellaria paradoxa* extracts using gallic acid as standard. ELVp represents the ethanol leaf extract of *Vitellaria paradoxa* while ESVp represents the ethanol seed extract of *Vitellaria paradoxa*.

The study of total phenol estimation using gallic acid as a standard showed that ethanol leaf extract of *Vitellaria paradoxa* has 83.46 ± 0.4167 µgGAE/mL while the ethanol seed extract of *Vitellaria paradoxa* has 68.88 ± 4.330 µgGAE/mL. From figure 6 below there was no significant difference between the leaf and seed extract of *Vitellaria paradoxa*. Furthermore the total flavonoid content was estimated from a standard quescestin curve showed the leaf extract of vitellaria paradoxa to be significantly higher in flavonoid when compared to the seed extracts as shown in figure 7.

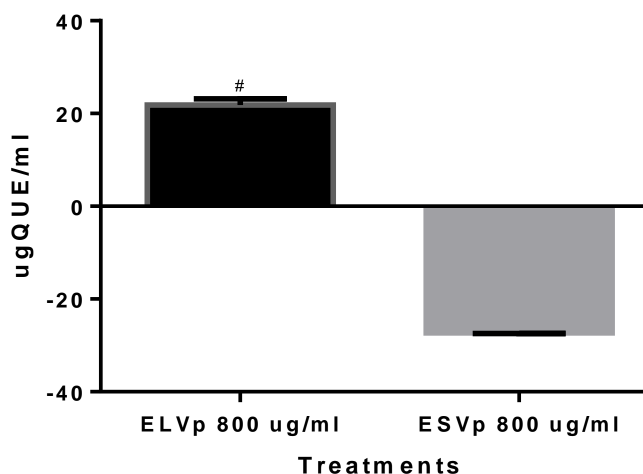

Figure 7:
Total flavonoid capacity of *Vitellaria paradoxa* extracts using quercetin as standard.
Where # represents significant when compared to ESVp treatment. ELVp represents the ethanol leaf extract of *Vitellaria paradoxa* while ESVp represents the ethanol seed extract of *Vitellaria paradoxa*.

The toxicity of the leaves and seeds extracts of Vitellaria paradoxa to *E.coli*PQ34 were investigated using the SOS chromotest. From the result various folds of dilutions of both extracts from 2 folds to 64 folds dilution was not genotoxic since the SOS induction factor was ≤ 1.5 however at the first concentration 800µg/ml (undiluted) for both leaf and seed extracts of *Vitellaria paradoxa* were inconclusive with SOSIF value of 1.5 and 1.6 respectively relative to the positive control 4-Nitroquinoline-1-oxide with value 4.1. Furthermore the positive control, 4-nitroquinoline-1-oxide SOS induction factor was ≥ 2.0 at 2 folds and 4 folds dilution as shown in figure 8

DISCUSSION

Widely distributed in plants are the secondary metabolites which primarily are used as adaptive measure for protecting the plant. It has been found that these metabolites called phytochemicals have a great potential in scavenging free radicals generated from endogenous and exogenous source (Olorunnisola *et al*., 2012; Seladi *et al*., 2014; Ehimwenma and Osarieme, 2014). Reactive oxygen species have been linked with a lot of disease conditions such as heart disease, inflammation, diabetes, cancer (Farombi *et al*., 2002). However the major mechanism that can be used to circumvent this menace are the antioxidant. In the present studies, the antioxidant activities of *Vitellaria paradoxa* of leaves and seeds were evaluated.

From the result we obtained the ethanol leaf extract of *Vitellaria paradoxa* has more antioxidant capacity than the seed extract with a 1.4 fold difference. This is in line with the finding by Neha *et al*., (2014) who showed the antioxidant capacity of the leaf extract of *Ocimum sanctum* (Holy Basil) to be higher than that of the seed extracts

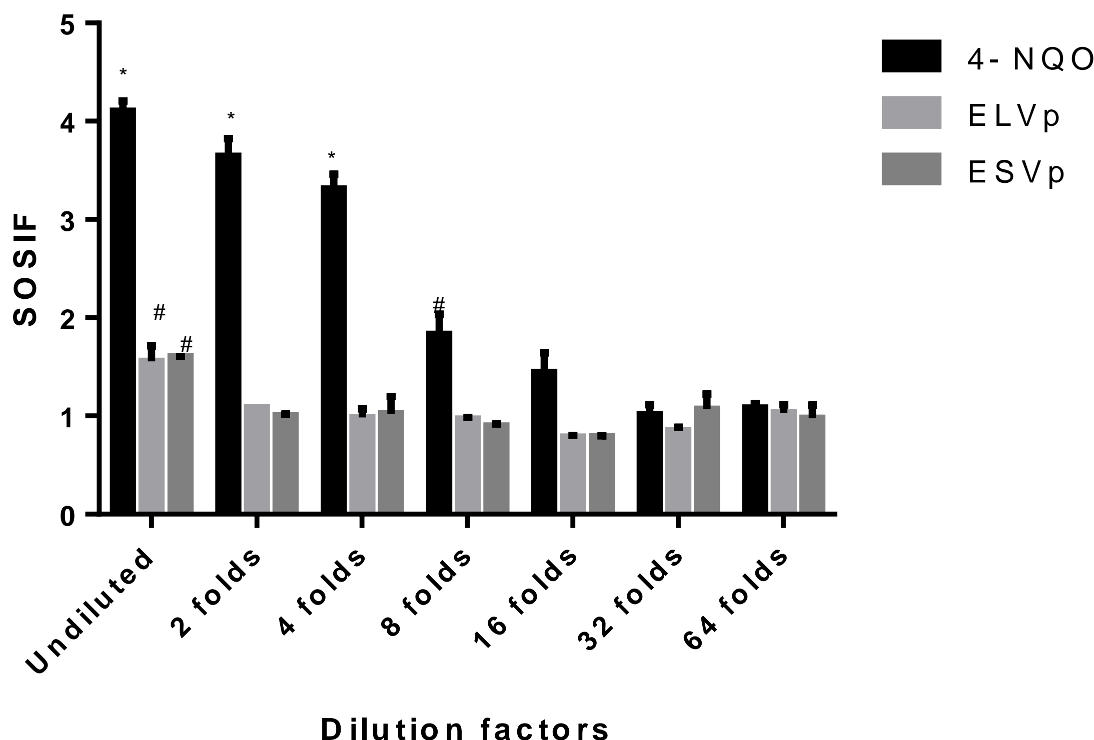

Figure 8:
Showing the genotoxic effect of *Vitellaria paradoxa* extracts and the positive control (4- nitroquinoline-1-oxide). Where * represents genotoxic since SOS inducing factor is ≥2 and #represents marginal genotoxic (inconclusive)

Although reductants are not necessary antioxidant however an antioxidant are reductant (Olugbami *et al*., 2015), hence the need to further assay for the reducing ability of *V. paradoxa* extracts. The presence of reductant in *V. paradoxa* caused the reduction of Fe^{3+} to Fe^{2+}. It is worthy to note the reducing power of both extracts of *Vitellaria paradoxa* is concentration dependent. Hence the higher the concentration the more the reducing power. This is in line with Olugbami *et al*., 2015 where the extract *Terminalia glaucescens* was concentration dependent. In addition our result support the study of Lin *et al*., (2014) who showed the leaf and stalk extract of *Perilla frutescens* to have a higher reducing power properties in comparison to the seed extract.

The stable DPPH scavenging model is widely used to assess the free radical scavenging ability of various samples (Ebrahimzah *et al*., 2008; Hamzah *et al*., 2014. In contrast to the result from Neha *et al*., (2014) and Lin *et al*., (2014) where both studies showed leaf extract of plant to have higher percentage inhibition for DPPH radical than seed, our study showed no significant difference the leaf and seed extract of *Vitellaria paradoxa*. In addition both extracts were had a lower % inhibition on DPPH as concentration increased when compared to ascorbic acid except at 1000 µg/ml.

Interestingly, the leaf extract of *Vitellaria paradoxa* was shown to be richer in total phenol content than the seed extract with a value of (83.46 ± 0.72 and 68.88 ± 7.50) µgGAE/ml respectively.. This follows the study by Neha *et al*., (2014) in which the leaf extract of *Ocimum sanctum* (Holy Basil) was higher in total phenol content than that of the seed extracts. However in the study of Zakizadeh *et al*., (2011) who accessed the in vitro antioxidant activity of the flower, seed and leaves of *Alcea hyrcana Grossh*, the seed was richer in total phenol than the leaf 68.9 ± 3.7 and 14.7 ± 0.9 mg gallic acid equivalent/g of extract powder, respectively.

Our result on total flavonoid content also support the leaf extract to be richer in flavonoid content than the seed with 21.83 ± 0.7631 for ELVp and -27.27 ± 0.1062 for ESVp. In biological system the antioxidant activities of phenolic compound and flavonoid is already established (Palacios, et al 2011; Olugbami *et al*., 2015), thus validating the extracts as radical scavengers.

The genotoxic activity of the extracts were assayed using the SOS chromotest a widely used assay for studying genotoxic and antigenotoxic activities of extracts/ constituents from medicinal plants. The *E. coli* PQ37 bacterial system is sensitive and dependable. It employs the error- prone DNA repair pathway also known as SOS response, a complex regulating network that is induced by DNA- damaging substance (Quillardet and Hofnung, 1982; Chaabane *et al*., 2012). From previous work it is known that a compound is classified as not genotoxic if the SOSIF remains ≤ 1.5, inconclusive if the SOSIF is between 1.5- 2.0 and genotoxic if SOSIF exceed 2.0 (Kevekordes *et al*.,1999; Fabiana *et al*.,2017). From our result the different concentration of both extracts added to the indicator bacteria were not genotoxic however at the first concentration 800µg/ml for both ELVP and ESVp extracts was inconclusive with SOSIF value of 1.5 and 1.6 respectively relative to the positive control 4-Nitroquinoline-1-oxide with value 4.1.

In conclusion, using *in vitro* antioxidant/ phytochemical assay methods the antioxidant/pytochemical properties of *Vitellaria paradoxa* leaf and seed extracts were compared. The study showed the leaf extract of *Vitellaria paradoxa* to have more antioxidant properties when compared to the seed extract. Various concentration of both extracts used for the SOS chromotest did not induce any genotoxicity however the both extract was marginally genotoxic at very high concentration of 800µg/ml. Therefore, the findings in this study distinctly showed the leaf of *Vitellaria paradoxa* as a good source of antioxidant and called for caution against over use of the plant for medicinal purpose.

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Conflicts of interest

There are no conflicts of interest declared.

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