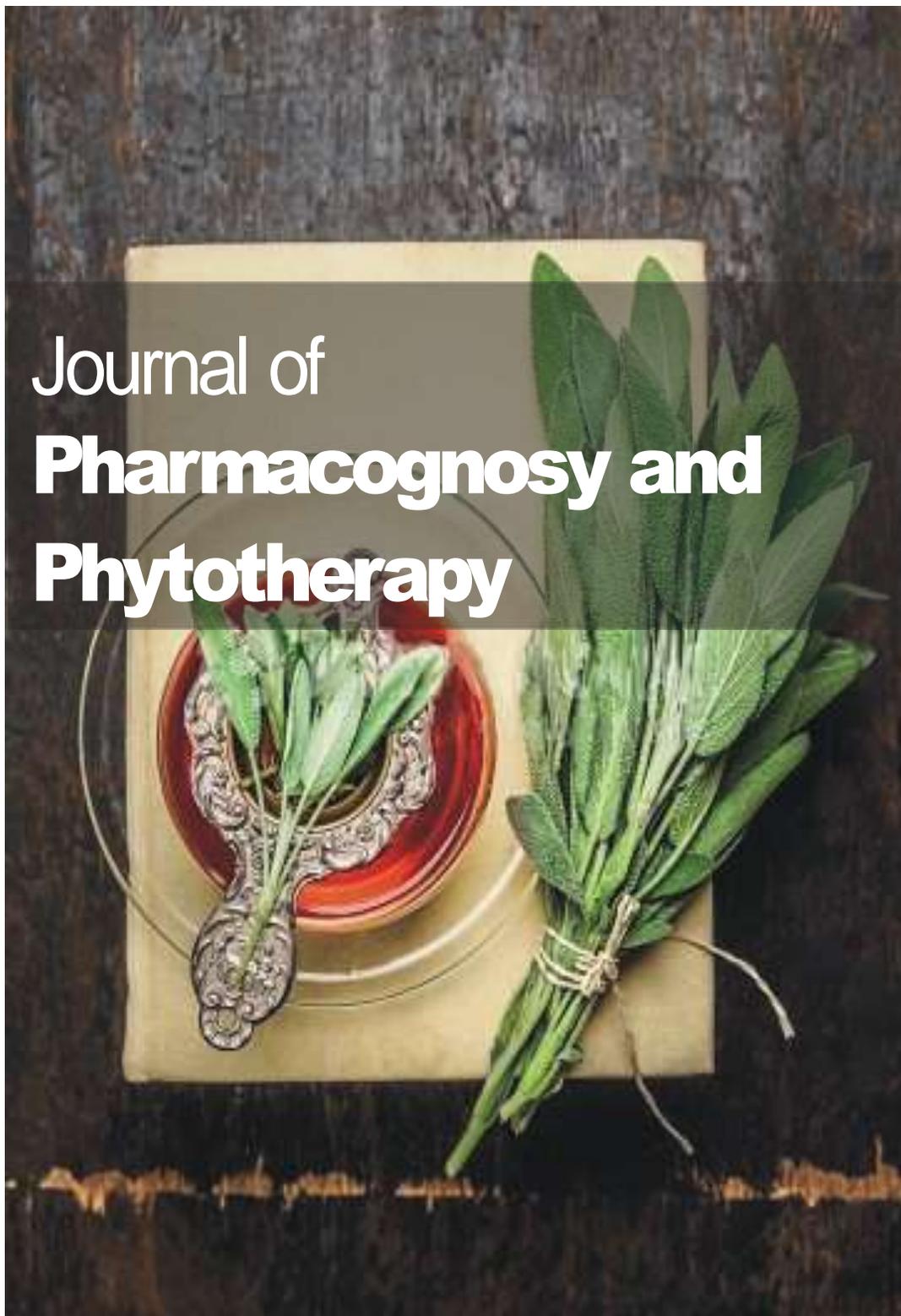


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# Journal of Pharmacognosy and Phytotherapy

Table of Contents: Volume 10 Number 7 July 2018

## ARTICLES

- Pulcandi a healing natural ash coal against gastric acidity: Scientific comprehension** 110  
Akpalo Edéfia, Aba Eklu Gudrun, Awaga Kwami Lumo and Aba Eklu Abatévi
- Comparative evaluation of the protective effect of leaf extracts of *Vernonia amygdalina* (bitter leaf) and *Ocimum canum* (curry) on acetaminophen induced acute liver toxicity** 116  
Michael Okey Enemali and Stanley Chidi Udedi

*Full Length Research Paper*

# **Pulcandi a healing natural ash coal against gastric acidity: Scientific comprehension**

**Akpalo Edéfia<sup>1,2\*</sup>, Aba Eklu Gudrun<sup>1</sup>, Awaga Kwami Lumo<sup>2</sup> and Aba Eklu Abatévi<sup>1</sup>**

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**As a coal, Pulcandi possesses the property of removing excess gastric and intestinal gas. Pulcandi powder has anti-diarrhea activity by decreasing intestinal fluid content and adsorbing microbes and toxins. The aim of this study was to demonstrate and to establish consequences of Pulcandi powder use on gastric acidity. For evaluating these consequences, it was investigated *in vitro* kinetic tests on acidity. These tests were followed by acidic dosages performed on Pulcandi aqueous extractions solutions. In this way, preparations were composed of 2.5 g powder mixed with 100 mL HCl acidic solutions. The study results revealed evident decrease of acidity with Pulcandi. However, the local concentration of protons impacted on this variation, which led to conclude that this coal do not increase pH when ulcers troubles don't occur. Infra-Red analyses (IR-ATR) were also performed on Pulcandi powder. The results evidenced presence of nitrate and bicarbonate ions neutralized protons. Moreover, essential minerals (Ca, Na, K, and Mg) present in Pulcandi powder could improve the coal properties and efficacy.**

**Key words:** Pulcandi, coal, extraction, acidity adsorption.

## **INTRODUCTION**

Gastric mucus is a whole of mucosa substances produced by cardial, pyloric and fundic cells. Mucus forms a double barrier of protection. This barrier prevents direct contact between stomach cells and gastric juice (HCl and pepsin). In fact, the pH at the surface of this barrier is 1.5 whereas it is 7 in deep zone. Cells producing gastric mucus, also produce important quantity of bicarbonate ions (HCO<sub>3</sub><sup>-</sup>) which neutralize locally protons ions. Then they have a chemical protective role (Murray et al., 2000).

Gastro-oesophageal reflux disease (GERD) is a

common disease in Western countries and the number of patients with GERD is increasing in Japan as well (Shinkai et al., 2014). Gastric ulcer is common gastrointestinal tracts disorder that affect about 10% of the world population. It is characterized by gastrointestinal tract bleeding, perforation and erosion of the mucosa wall due to imbalance between aggressive and defensive factors. Aggressive factors are acid, pepsin and *Helicobacter pylori* (Adefisayo et al., 2017).

Gastric acid is known to cause marginal ulcers, situated in the small bowel just distal to the upper anastomosis

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(Bekhali et al., 2017). Defensive factors are: mucin, prostaglandin, bicarbonate, nitric oxide, mucosal blood flow and growth factors (Adefisayo et al., 2017). Proton pump inhibitors (PPIs) are known to be the most effective drugs that are currently available for suppressing gastric acid secretion. However, other studies have reported that PPIs may not be able to render stomach achlorhydric, and have identified a phenomenon of increasing gastric acidity at night in individuals receiving a PPI twice daily (Shim et al., 2017).

Secular, ancestral and traditional knowledge reported in literature demonstrated that coal suppresses excess gastric and intestinal gas, neutralizes microbes and toxins. It heals diarrhea and gastroenteritis. Coal is a black color solid combustible matter with plant origin containing high proportion of carbon. It is a fine, odorless and smooth powder. Active or activated coal obtained by pyrolysis has a porous structure. Depend on the transformation temperature, the obtained product conserves initial properties or acquires new properties (Avom et al., 2001).

Among the different methods to prevent intestinal absorption of ingested poisons, charcoal is the easiest to administer, has few side effects and is preferred by most volunteers (Daniel et al., 1988). Activated charcoal is a method of gastric decontamination that is now being recommended for patients who have ingested potentially toxic amounts of poison up to 1 h previously (Christophersen et al., 2002). Activated charcoal has the ability to adsorb a wide variety of substances. This property can be applied to prevent the gastrointestinal absorption of various drugs and toxins, and to increase their elimination, even after systemic absorption (Neuvonen et al., 1988).

Clinical studies in 2002 recommended gastric lavage or gastric lavage followed by activated charcoal which may be replaced by charcoal alone for treating (Christophersen et al., 2002). Activated charcoal (AC) is a non-selective and highly effective adsorbent (Jia et al., 2013). For example, significant *in vitro* binding of colchicine to AC suggests that AC has a role in decontamination of patients after ingestion of *Gloriosa superba* (Zawahir et al., 2017).

Gastric acidity is different in various disease states and its measurement may be useful in the diagnosis and treatment of these diseases. Gastric acid measurement may also be useful in assessing the effectiveness of new antisecretory drugs (Sinkai et al., 2014). Pulcandi is a black, fine, odorless and smooth powder obtained by fodder crops calcinations. This later contains mineral such as potassium, magnesium or sodium. It is not well known how Pulcandi powder interacts with gastric biomolecules to heal ulcers and gastro-enteritis. A special interest has been shown by scientists regarding the popular testimonials on the product.

The aim and motivation of this study was to establish scientific comprehension of the efficiency of this product,

and to show its bioactivity.

## MATERIALS AND METHODS

### Chemicals

The product Pulcandi was produced by Centre Omnitherapeutique Africain (COA). (African Omnitherapeutical Center). Chlorhydric and sulfuric acids were purchased from Prolabo (Togo) and phenolphthalein from Sigma (France). Distilled water was obtained from laboratory of Biochemistry Department (Universite de Lome). Experimentations were performed in the biochemistry laboratory of COA. Infra-Red analyses were performed in CRISMAT Laboratory (France).

### *In vitro* kinetic tests on acidity

50 mL of an aqueous Pulcandi solution with a concentration corresponding to adult posology, meaning 10% in water, was added to 150 mL of an acetic acidic solution (pH=1) mimicking gastric acidity. The mixture was shaken for 100 min with a Titramax 100T orbital shaker at 600 rpm. The pH values were then measured each 5 min with a pH-meter. Each experiment was done in duplicate, and the mean value was recorded.

### Aqueous extractions and acidic dosages

To a 0.1M HCl solution, 2.5 g /100 mL Pulcandi powder was added. The mixture was shaken for 1 h with a Titramax 100T orbital shaker (600 rpm) at 25°C. This solution was filtered with a Whatman filter paper and 25 mL was dosed. To the filtered solution 2.5 g /100 mL Pulcandi powder was once again added. The obtained solution was shook for 1 h with a Titramax 100T orbital shaker at 600 rpm at 25°C, filtered with a Whatman filter paper and 25 mL was dosed. The same process was done a third time. To perform each step dosage, 1 mL of phenolphthalein (1% in acetone) was added and the titration was done with a 0.1 M NaOH solution. The mixture was gently stirred with an Ikamag magnetic stirrer until neutralization occurred. Each experiment was done in duplicate and the mean value was recorded (Table 1).

### Infra-red analysis

Infra-red spectra of Pulcandi powder was registered from 2000 to 600  $\text{cm}^{-1}$  on a spectrophotometer IR Tensor 27 with an ATR reflexion module.

### Minerals composition and concentration

Minerals elements concentration (Ca, K, Mg, Na) were determined by absorption of spectrophotometry. For this, Pulcandi powder was mineralised by 1.5mL nitric acid. The mixture was then heat until a viscous liquid was obtained, and then distilled water was added. The solution is filtered and diluted. Mineral element concentration determination was done with an atomic absorption spectrophotometer AA Solar, Tehrmo Electron Corporation (Germany).

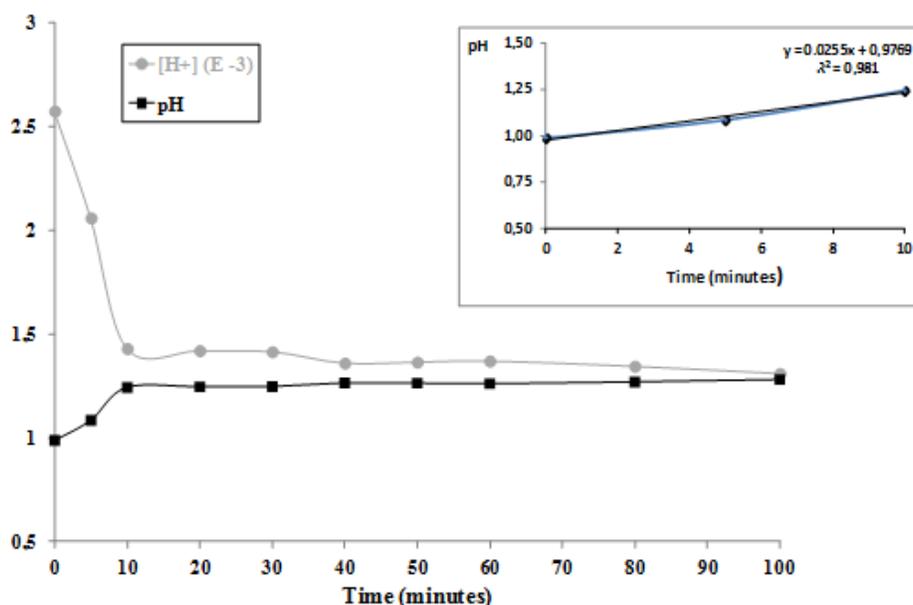
## RESULTS

### *In vitro* Pulcandi kinetic tests on acidity

Figure 1 shows an increase in pH from 0.99 to 1.28 pH

**Table 1.** Extractions and dosages processes.

Steps	Mixing	Filtrate	Dosage
1: Initial solution V = 300 mL [HCl] = 0.1 M	V = 300 mL mPulc = 7.5 g Concentration : 2.5g /100mL Shaking : for 1H at 600 rpm Temperature: 25-30°C	No. 1	N°1 Duplicate V = 25 mL V <sub>NaOH</sub> = 13.725 mL
2: Filtrate N°1 V = 200 mL	V = 200 mL mPulc = 5 g Concentration : 2.5g /100mL Shaking : for 1H at 600 rpm Temperature: 25-30°C	No. 2	N°2 Duplicate V = 25 mL V <sub>NaOH</sub> = 9.45 mL
3: Filtrate N°2 V = 100 mL	V = 100 mL mPulc = 2.5 g Concentration : 2.5g /100mL Shaking : for 1H at 600 rpm Temperature: 25-30°C	No. 3	N°3 Duplicate V = 25 mL V <sub>NaOH</sub> = 5.1 mL

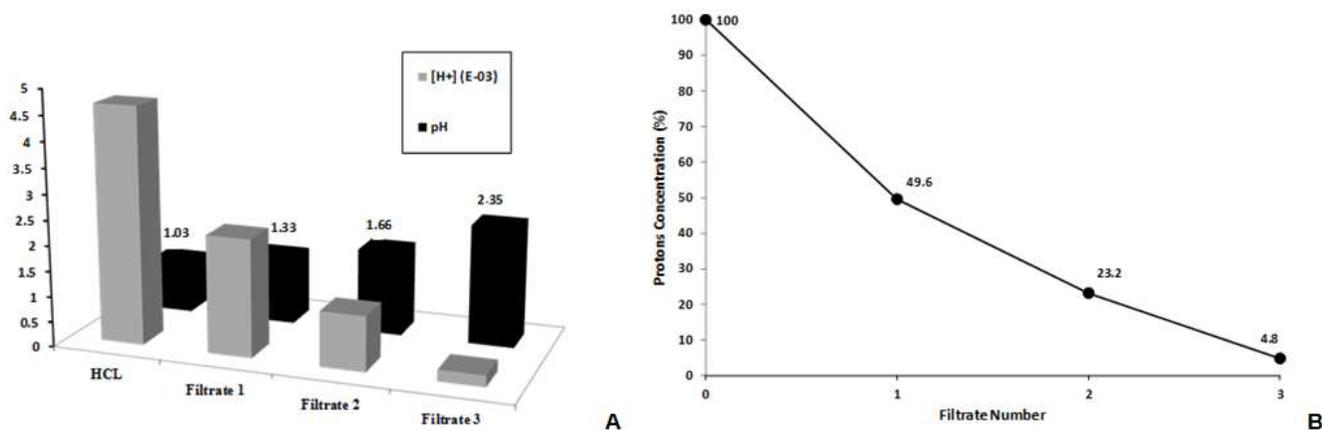
**Figure 1.** pH (■) and concentration of proton ions (●) evolutions of an acidic solution added with Pulcandi.

units at a kinetic of  $25.5 \times 10^{-3} \text{ unit} \cdot \text{min}^{-1}$  within the earlier 10 min. Afterwards, no more variation was observed. The trend went upward from 0 to 10 min with  $R^2 = 0.981$  which indicates an initial rate ( $25.5 \cdot 10^{-3} \text{ unit} \cdot \text{min}^{-1}$ ) followed with stagnation. This increase in pH values reflected in contrast a fast decrease (50%) of acidity from  $2.6 \cdot 10^{-3} \text{ M}$  of protons to  $1.3 \cdot 10^{-3} \text{ M}$  of protons in the presence of Pulcandi powder. This phenomenon was observed also within the earlier 10 min. This variation of 0.29 units of pH corresponded to a difference of  $5.06 \times 10^{-2}$

M of protons. This result was confirmed by a continuous dosage of a 0.1 M HCl solution (data not shown).

#### Preparation of aqueous extractions and acidic measurements

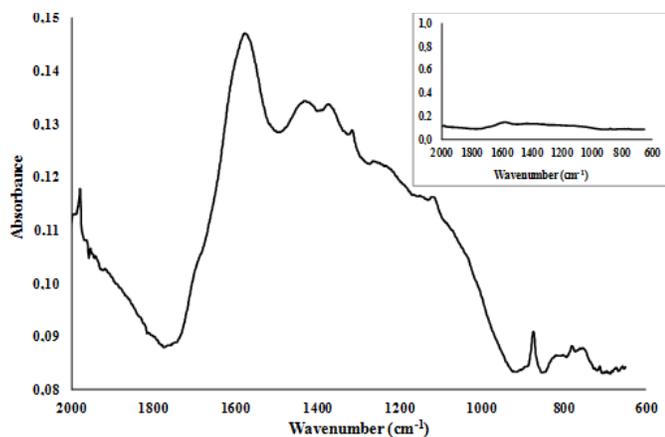
The primary processing of the sample was carried out according to the steps shown in Table 1. The working concentration of 2.5 g / 100 mL was used along the three



**Figure 2.** (A) Dosages of an acidic solution added with three fold Pulcandi (Protons concentrations and pH values); (B) Dosages of an acidic solution added with three fold Pulcandi (Variations of protons concentrations vs filtrate number).

**Table 2.** Relation between Pulcandi powder mass and quantity of protons adsorbed.

Filtration	Quantity of protons for 2.5 g of Pulcandi powder	Quantity of protons per g of Pulcandi powder (mol/g)
Filtrate 1	2.35	0.94
Filtrate 2	1.23	0.49
Filtrate 3	0.85	0.34



**Figure 3.** IR-ATR spectrum of Pulcandi powder.

times extraction. Results of these aqueous extractions acidic dosages (Figure 2A) highlight a progressive decrease of protons when filtration was on. In the same time, pH values evolve conversely and were doubled. Figure 2B highlights the fact that with the first dose of Pulcandi powder (2.5 g), 51.4% of protons were already adsorbed. The second dose of the same mass (2.5 g) adsorbed this time 26.4% of protons ions. Finally, the third dose had led to 18.4% adsorption. The variations were similar for a second sample (data not shown). To

elucidate this phenomenon, we searched to determine the molar quantity of protons adsorbed by gram of Pulcandi powder at each step of filtration. Results are presented in Table 2. The quantity of acidic ions per gram of Pulcandi powder varied from 0.94 mol/g to 0.49 mol/g and from 0.49 mol/g to 0.34 mol/g. Infra-Red spectra of Pulcandi powder were very difficult to obtain (Figure 3). We tried many times without any result (Transmittance = 100%). We decided then to reduce the scale end enlarge the spectra measuring in absorbance. NO<sub>2</sub> group was determined at 1575 cm<sup>-1</sup> which was confirmed at 1374 cm<sup>-1</sup>. At 1431 cm<sup>-1</sup>, the corresponding group was OH in carboxylic acids. Other signals were too weak to analyse.

### Mineral components of Pulcandi

As Pulcandi powder is an ash coal, we investigated the presence of essential minerals. Mineralisation analyses revealed the presence of Calcium, Natrium, Potassium and Magnesium respectively at 1445, 60, 2150 and 251 mg% (Table 3).

### DISCUSSION

Different analyses were done successively to achieve and understand the mechanism of action of Pulcandi powder: aqueous extractions, acidic dosages, Infra-Red

**Table 3.** Essential minerals present in Pulcandi powder.

Essential minerals	Pulcandi contents (mg%)
Calcium (Ca)	1445
Natrium (Na)	60
Potassium (K)	2150
Magnesium (Mg)	251

analyses and mineralisations.

The pH increasing of acidic solutions added with Pulcandi powder during the earlier 10 min (Figure 1) at a rate of  $25.5 \times 10^{-3}$  unit/min, allowed to confirm patients' observations on Pulcandi powder. In fact, patients' observations led to beneficial effect after 20 min including a light feeling of heartburn during the first 10 min.

It is necessary to take into account *in vivo* conditions when applying *in vitro* digestion methods, in order to maximally reproduce them. The gastric phase is performed with HCl or HCl-pepsin under fixed pH and temperature conditions, for a set period of time (Alegria et al., 2015). This was the reason this study was conducted with HCl acidic solutions at 0.1 M. The three steps corresponded to Pulcandi posology, that is, one teaspoon (2.5 g) with water (100 mL) 3 times a daily.

From the study observations, results presented in Figure 2A and B demonstrated drastic diminution of protons concentration from 100 to 4.8%. These variations are correlated with 51.4% protons adsorption, 26.4% of protons adsorption and finally 18.4% adsorption respectively after the first, the second and the third dose taken. The variations were similar for a second sample (data not shown).

Biochemical explanations are, on the one hand, parietal cells of the gastric mucosa secrete chlorhydric acide (HCl) which was neutralized by the mucus composed of bicarbonate ions ( $\text{HCO}_3^-$ ) (Murray et al., 2000). When *H. Pylori* infection occurs, the mucous cells are deteriorated preventing the regulation of HCl secretion. The mucous is then damaged leading to ulcers. In another hand, we also know that plant ashes are essentially composed of bicarbonate ions ( $\text{HCO}_3^-$ ) which are easily dissolved in water (Javillier, 1959). More, active coal was used in medicine as chelating product in many cases of intoxications. Then acidity adsorption property of Pulcandi powder ancestrally known and confirmed by the study results, is due to similar role of Pulcandi powder as mucus. The role of Pulcandi powder is to achieve quickly sufficient adsorption of protons.

To confirm the identity of the component that could neutralize protons, infra-red analyses were done on Pulcandi powder. Spectrum in Figure 3 (The profile of a second sample was the same. Data not shown) shows the presence of  $\text{NO}_2$  group in aliphatic nitro compounds and OH in carboxylic acids which could come from carbonic acid. These results show that this coal

possesses without doubt nitrite ( $\text{NO}_2^-$ ) and / or nitrate ( $\text{NO}_3^-$ ) ions as well as bicarbonate ions ( $\text{HCO}_3^-$ ). We then strongly suppose that nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ) ions frequently present in plants ashes came from atmospheric nitrogen (N). They could also have been produced during the transformation of Pulcandi powder since coal combustion produces nitric oxide (Lehninger, 1982).

Indeed, ashes represent comparatively to the real chemical composition of living, something artificial. It can be easily imagined that, in a certain tissue, salts provided from organic acids saturation by minerals basis; the organic part of such molecules will undergo the combustion and bases will subsist, generally in carbonate forms (Javillier, 1959). Then, these ions present in Pulcandi powder will neutralize protons in acidic solutions. They play defensive roles (Adefisayo et al., 2017).

Table 2 shows Pulcandi powder contents essential minerals: calcium, natrium, potassium and magnesium. In 2013, a team of scientists demonstrated that the phosphate-binding ability of calcium chloride was improved by AC in intestinal fluid (Jia et al., 2013). Potassium ions are implicated in proton pump mechanism (Murray et al., 2000) and their concentration (2150 mg%) is the double of the concentration presents in lentils (1200 mg%), a high potassium content food. This study was deepened by determining the quantity of protons adsorbed per gram of Pulcandi powder at each step of the process. The values obtained and presented in Table 2 allowed to estimate the efficiency of Pulcandi powder. We remarked that less and less protons were adsorbed as filtrations go by. This highlights that the more higher the local protons concentration was, the more these ions are adsorbed in Pulcandi powder.

In contrast, in a low protons concentration environment, protons did not "knock" themselves to enter the pores of the calcinated powder. A maximum efficiency of adsorption was obtained after the first filtration (approximately 51.4%). The adsorption phenomenon observed here is first of all the probability of access to the charcoal pores after three doses. However, repeated doses of charcoal also reduce the risk of desorption from the charcoal-toxin complex as the complex passes through the gastrointestinal tract (Neuvonen et al., 1988). Then, it is not a question of saturation of Pulcandi powder in our study as Figure 1 could suggest, meaning a given mass could not adsorb more than a determined quantity of protons. It is a question of protons capacity to hit powder particles or eventually being in competition with water molecules ( $\text{H}_2\text{O}$ ). Gastric acid suppression was reported to increase the risk for development of drug hypersensitivity reactions. These consequences of anti-ulcer drug intake associated with direct influence of these drugs on immune responses. On the other hand reduction of gastric acidity leads to impaired gastrointestinal protein degradation (Untersmayr, 2015).

Using Pulcandi, a biocompatible natural ash coal prevents those chemical drugs consequences. This powder is then of practical interest.

## Conclusion

The main point of this study was the evident diminution of acidity when Pulcandi powder was added. However, acidity adsorption was not systematic and drastic. As it depends on local concentration of protons, anyone who takes Pulcandi powder without suffering of gastric acidity ulcers will not be subject to a sudden gastric juice alkalisation. This last phenomenon will allow a bacterial proliferation leading to other troubles. Such biochemical analysis lead to Pulcandi powder generated promising preclinical and clinical results for treatments of gastric ulcers with Pulcandi powder. The study results have shown that Pulcandi is highly active in protons suppression. It would be interesting to continue our experimentations focus on magnesium and potassium which are important ions implicated in psychosomatic gastric ulcers.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# **Comparative evaluation of the protective effect of leaf extracts of *Vernonia amygdalina* (bitter leaf) and *Ocimum canum* (curry) on acetaminophen induced acute liver toxicity**

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The quest for alternate medicine in preventing and combating hepatotoxicity led to the screening of aqueous and ethanol extracts of dried leaves of *Vernonia amygdalina* Delile (bitter leaf) and *Ocimum canum* Linn (curry leaf) for phytochemicals and *in vitro* antioxidant properties. Some rats were pretreated with the extracts and later administered with 2g/kg of body weight single dose of acetaminophen and the hepatoprotective effect of the extracts was determined by assessing the liver function, antioxidant enzyme activities and histological status of their livers, using standard biochemical methods. The extracts were rich in bioactive compounds and showed concentration-dependent variation in *in vitro* free radical (DPPH) scavenging activity. A 400mg/kg of body weight per day pretreatment (for seven days) with the extracts gave hepatoprotection to the rats. This was evidenced in the reduction of the activities of alanine aminotransferase (ALT) by 43% in aqueous extract of *O. canum* to 92% in *V. amygdalina* and marked increase of the serum albumin concentration. Aqueous leaf extract of *V. amygdalina* caused the highest increase in GPx activity while ethanol leaf extract of *O. canum* gave the highest (350%) increase in superoxide dismutase (SOD) activity, compared to the negative control. Also the lobular architecture of the hepatocytes was preserved. *V. amygdalina* and *O. canum* have important role in medicine as they contain substances that scavenge free radicals, stimulate activities of antioxidant enzymes and preserve the liver architecture in occasion of acetaminophen-induced liver toxicity.

**Key words:** *Vernonia amygdalina*, *Ocimum canum*, hepatoprotection, oxidative stress, free radical-scavenging, hepatocytes.

## **INTRODUCTION**

Acetaminophen (APAP) is an analgesic and antipyretic substance used in the production of the drug paracetamol. Although safe at therapeutic doses, APAP had been

found to cause severe liver injury (Vidhya and Bai, 2012; Erica and Emily, 2014).

It is metabolically activated by cytochrome P<sub>450</sub>

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enzymes to a reactive metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI), that depletes glutathione (GSH) and covalently bonded to protein, and repletion of glutathione (GSH) prevents the toxicity (James et al., 2009).

NAPQI is formed by a direct two-electron oxidation of acetaminophen and can be detoxified by glutathione (GSH) to form an acetaminophen-GSH conjugate (Dahlin et al., 1984). After a toxic dose of acetaminophen, total hepatic GSH is depleted by as much as 90%, and as a result, the metabolite covalently binds to cysteine groups on protein, forming acetaminophen-protein adducts (Mitchell et al., 1973 ; Jack et al., 2009). Depletion of GSH which is an intrinsic antioxidant is capable of regeneration of reactive oxygen free radicals and hepatocellular fatty regeneration with centriolobular necrosis of the liver. Over production of reactive oxygen species (ROS) can damage cellular biomolecules like nucleic acids, proteins, lipids, carbohydrates and enzymes resulting in several diseases (Halliwell and Guteridge 1999). Living systems have specific pathways to overcome the adverse effects of these damages but sometimes these repair mechanisms fail to keep pace with such deleterious effects (Nilsson et al., 2004).

In chronic liver diseases caused by oxidative stress (alcoholic and non-alcoholic fatty liver diseases, drug- and chemical-induced hepatic toxicity), the antioxidant drugs such as silymarin can have beneficial effect (Feher and Lengyey, 2017). Silymarin is the active ingredient in the branded drug, Sylibon 140 (a known hepatoprotective drug), manufactured by Micro Laboratory Ltd, India. Silymarin has cyto-protective activity mediated by its anti-oxidative and radical-scavenging properties, (Křena and Walterovab, 2005).

Focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants for treatment purposes or for the production of drugs (Dahanukar et al., 2001; Olamide and Mathew, 2013; Udochukwu et al., 2015). Their use in ethnomedicine for the management of ailments stem from the presence of phytochemicals (Aja et al., 2010). In Nigeria, leafy vegetables contribute greatly to the nutritional and medicinal needs of the people as staples, flavours, condiments, spices, drinks and beverages (Mbang et al., 2008; Cheveller 1996).

Methanol extracts (Saud et al., 2013) and ethanol extracts (Ladipo et al., 2010) of vegetables could be used in treating diseases caused by microorganisms and they boost the concentration of Red Blood Cell in experimental animals, (Ezekwe et al., 2013). Pharmacological studies have demonstrated hepato-protection, antioxidant and anti-inflammatory activities supporting the traditional uses of some leafy vegetables (Perez, 2016; Ahmed, 2016). These effects have been attributed to polyphenols and flavonoids in plant extracts which improve the functionality of the antioxidant system of the test rats (Rice-Evan et al., 1996; Edeoga et al., 2005; Sudha et

al., 2011; Imaga and Bamigbetan 2013; Alamgir et al., 2016).

*Vernonia amygdalina*, a member of the Asteraceae family, is a small shrub that grows in tropical Africa. It typically grows to a height of 2 to 5 m (6.6 to 16.4 ft). The leaves are elliptical and up to 20 cm (7.9 in) long. Its bark is rough (Ijeh and Ejike, 2011). *V. amygdalina* is commonly called bitter leaf in English because of its bitter taste. The cooked leaves are a staple vegetable in soups and stews of various cultures throughout equatorial Africa (Egedigwe, 2010; Kokwaro, 2009). African common names include; Grawa (Amharic), Ewuro (Yoruba), Etidot (Ibibio), Onugbu (Igbo), Ityuna (Tiv), Oriwo (Edo), Etidot (Cross River State of Nigeria) Chusar-doki (Hausa), Mululuza (Luganda), Labwori (Acholi), Olusia (Luo) and Andndoleh (Cameroon) (Egedigwe et al., 2010; Kokwaro et al., 2009).

*Ocimum canum* belongs to the Lamiaceae family and it is native to the African continent and grows to a height of 2 feet. It is also known as the African basil, commonly known as curry among the native communities of Nigeria, with a distinct mint flavour and scented flowers. *O. canum* is grown for its medicinal and culinary value and it is highly useful in treating various types of diseases and in lowering blood glucose levels, especially in type 2 diabetes and treat cords (Kokwaro et al., 2009).

Huge quantities of these plants *O. canum* Linn (curry leaf) and *V. amygdalina* Delile (bitter leaf) are consumed all over Nigeria for flavouring and spicing of various types of food and as medicine for different ailments. Available information on their hepatoprotective property is scanty. Therefore, it is expedient to establish their hepatoprotective potentials of aqueous and the ethanol leaf extracts of these plants against injuries that may be caused by xenobiotics especially the commonly abused drug, paracetamol. This will clarify if their use can aid specific pathways, which work to overcome the adverse effects of damages caused by these xenobiotics, to keep pace with such deleterious effects.

## MATERIALS AND METHODS

### Pant materials, silymarin and acetaminophen

The plant materials were leaves of *V. amygdalina* and *O. canum*, the drug, Acetaminophen was a research support from Emzor Pharmaceutical Ltd, Lagos while silymarin is a branded drug (Sylibon 140) from Micro Laboratory Ltd, India.

### Sample collection and preparation

Plant materials were harvested from two vegetable farms in Keffi, Nasarawa state, North Central Zone of Nigeria. The plants were identified by a Taxonomist Dr. B. O. Aziagba of the Department of Botany, Nnamdi Azikiwe University, Awka (NAUH). The *V. amygdalina* was assigned voucher number NAUH 47A and the *O. canum* was assigned NAUH 144A and both were deposited at the University herbarium. The leaves were rinsed in water to remove

dust and sand particles, and then dried under room temperature for fourteen (14) days. The dried leaves were then grounded into powder using electric blender. Ethanol and water were separately used to extract the bioactive ingredients from the leaves.

### Preparation of extracts

Bioactive compounds were extracted by soaking 100 g of the plant materials in 500 mL of absolute ethanol (that is, ratio 1:5; weight to volume) for 48 h. The extract was filtered using muslin cloth and then concentrated by heating at 30°C in a water bath and stored in airtight containers at 4°C. The aqueous extracts of the plant samples were prepared by soaking 100g of the powdered samples in 500mL of distilled water (that is, ratio 1:5; weight to volume) for 48 h. The extracts were filtered using muslin cloth and then concentrated by freeze drying and stored in airtight containers at 4°C.

### Animal models

Forty two male Wister albino rats weighing between 120 to 140 g were used for the study. These rats were purchased from the animal house of the National Veterinary Research Institute (NVRI), Vom in Plateau state. They were housed in clean, well ventilated metal cages in the animal house of the Department of Biological Sciences (Zoology unit), Nasarawa State University Keffi. The animals were kept under 24 h light/dark cycling. They were allowed access to unlimited food and water supply and allowed to acclimatize for three (3) weeks before the commencement of the study. All the animals were marked for identification, and their respective weights recorded. The animals were first fed with the chow (feeds) and intubated with the plant material.

### Administration of extracts and the drugs to the animals:

The albino rats were divided into seven (7) groups of six (6) animals each. The group 1 (untreated group) received feed and water only, group 2 (the standard control) received feed, water and a pretreatment with 400mg/kg of body weight of Silymarin. Group 3 (negative control) received feed and water, while the groups 4-7 (test groups) received feed, water and pretreatment with 400mg/kg of body weight of either ethanol or aqueous leaf extracts of *V. amygdalina* or *O. canum* for seven days. On the eighth (8) day, the animals were fasted for up to seven hours, followed by the induction of hepatotoxicity in the animals of groups 2 to 7 by oral administration of 2g/kg of body weight acetaminophen.

### Animals sacrifice, collection and preparation of samples

9 h after intoxication, the animals were made unconscious by exposure to chloroform in an enclosed container, as described by Ekor et al. (2006). Incisions were quickly made into the animals' cervical region with the aid of sterile blades and blood samples collected into plain tubes. Serum was collected as supernatant after centrifuging the clotted blood in a HSC (1000 to 4000rpm) bench centrifuge at 3000 rpm for 10 min.

### Preparation of liver homogenate

After bleeding, the livers were carefully removed, trimmed of extraneous tissues and rinsed in ice-cold 1.15% KCl. The livers were then blotted dry, two grams (2 g) was weighed and homogenized in 8 ml of ice-cold phosphate buffer (100mM, pH 7.4).

The homogenate was then centrifuged first at 6,000 rpm for six minutes (6 mins) to remove nuclear debris after which the supernatant were centrifuged at 10,000 rpm for twenty minutes (20 mins) to obtain the post-mitochondrial supernatant (PMS), using a refrigerated centrifuge. This was used for the assay of the antioxidant enzymes (Super Oxide Dismutase, Catalase and Glutathione Peroxidase).

### Biochemical analysis

The biochemical analyses were carried out using standard methods. Portions of the concentrated extracts were used for phytochemical screening using standard procedures of the Association of Analytical Chemist (2006) to identify the constituents as also described by Odebiyi and Sofowora (1990 1978), Fadeyi (1983), Sofowara (1993), Trease and Evans (1989) and Harborne (1973). The evaluation of free radical scavenging activity of the plant extracts against DPPH radical was done by a slightly modified spectrophotometric method previously described by Afolayan et al. (2014). The serum Alkaline Phosphatase activities of the experimental animals were estimated using the method of King (1965b) while the activities of Aspartate aminotransferase and Alanine aminotransferase were carried out using the King (1965a) method. The total protein was estimated using the colorimetric method of Lowry et al. (1951) where the peptide bonds of proteins react with  $\text{Cu}^{2+}$  in alkaline solution to form a coloured complex. Malloy and Evelyn (1937) method which has a principle that states that in an aqueous solution only direct bilirubin (DB) reacts, was used to determine total bilirubin. Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by Sun and Zigma (1978). The catalase activity was determined according to the method of Beers and Sizer as described by Usoh et al. (2005) by measuring the decrease in absorbance at 240nm due to the decomposition of  $\text{H}_2\text{O}_2$  using the ultra violet (UV) spectrophotometer. The method of method of Lawrence and Burk (1976) was used for the determination of glutathione peroxidase (GPx) activity. Finally, the histological study on the liver tissues was carried out using the Haematoxylin and Eosin stain as described by Bancroft et al. (2013).

### Statistical analysis

Data were expressed as means  $\pm$  standard deviation (SD). The statistical tools used for the analysis was one way analysis of variance (ANOVA) and the post hoc Newmann Keul's multiple comparisons test. The computer software utilized were Microsoft excel 2016 edition and statistical package for social sciences (SPSS) 16.0 for windows. Differences between means were considered significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

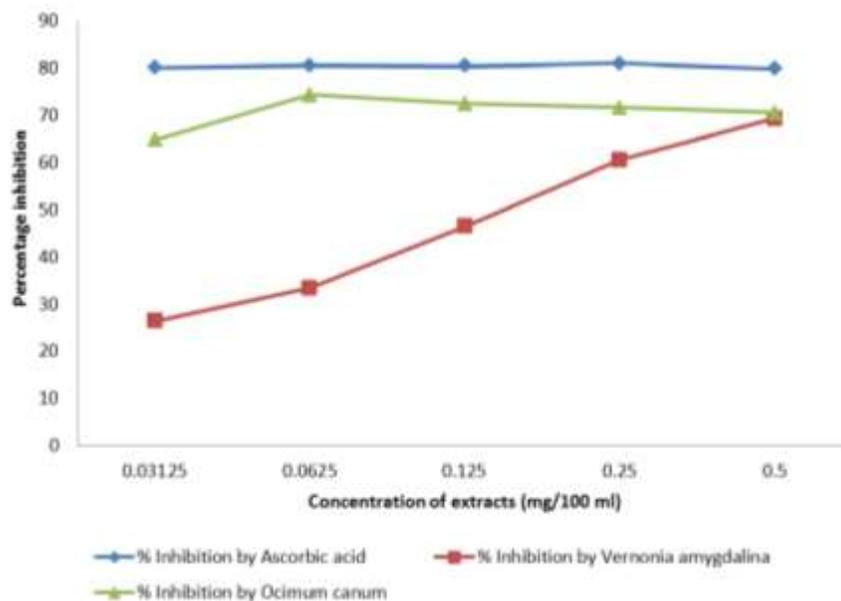
The extracts contain varying types of phytochemicals. All the extracts contain tannin and glycosides; while only ethanol leaf extract of *V. amygdalina* contained alkaloid. Saponins are contained in all the extracts, but the ethanol leaf extract of *O. canum*. All the extracts, except the aqueous leaf extract of *O. canum* contain phenols (Table 1).

The aqueous leaf extract of the *V. amygdalina* had a direct variation of *in vitro* free radical (DPPH) scavenging property with increase in concentration of the extract from 0.03 to 0.5mg/l (26.39 to 69.31%) while *O. canuman*

**Table 1.** The qualitative phytochemical compositions of aqueous and ethanol leaf extracts of *Vernonia amygdalina* and *Ocimum canum*.

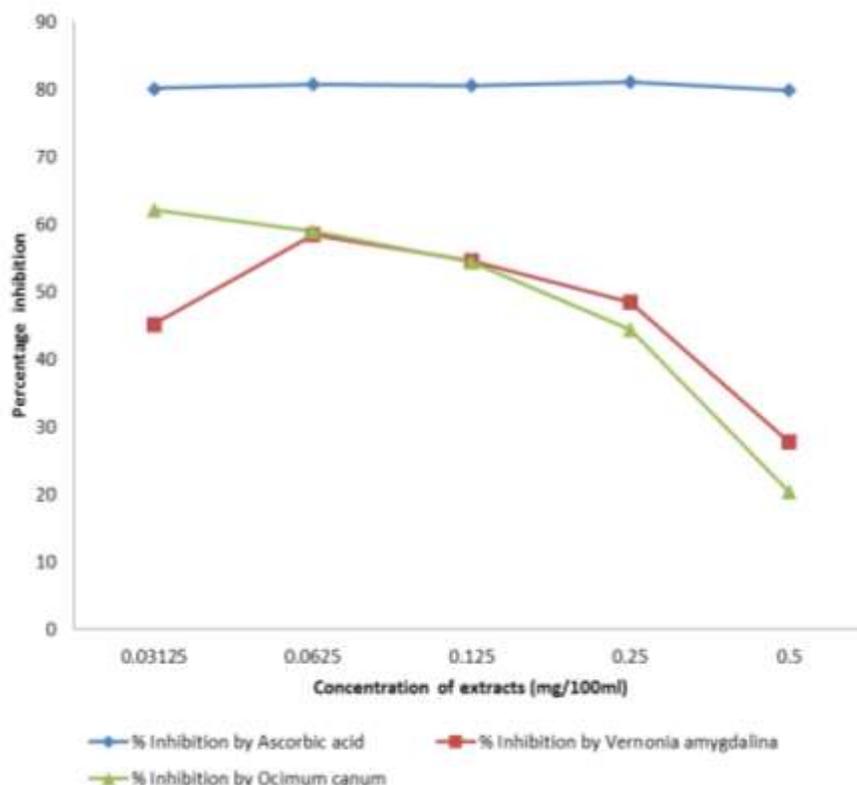
Parameters	<i>Vernonia amygdalina</i>		<i>Ocimum canum</i>	
	Aqueous	Ethanol	Aqueous	Ethanol
Alkaloid	-	+	-	-
Tannin	+	+	+	+
Phenols	+	+	-	+
Cardiac glycosides	+	-	-	+
Triterpenoids	+	+	-	+
Sterols	+	-	-	-
Terpenoids	-	+	+	+
Saponins	+	+	+	-
Balsam	-	+	-	+
Cardenolides	-	-	-	-
Resins	-	-	-	-
Phlobatanins	+	-	+	+
Glycosides	+	+	+	+

+ ... indicates presence; - ... indicates absence.

**Figure 1.** Comparing the free radical, (DPPH), scavenging activity of aqueous extracts of *V. amygdalina* and *O. canum* using ascorbic acid as positive control. Values are means of triplicate results of the inhibition.

increase from 64.86% at 0.03mg/100ml to 74.31% at 0.06mg/ 100ml, and gradually dropped 70.56% at a concentration of 0.5mg/ 100ml. Based on the concentrations used in the study, *O. canum* had its highest *in vitro* antioxidant activity at 0.06mg/ 100ml, *V. amygdalina* had its highest activity at 0.5 mg/ 100ml (Figure 1). The ethanol leaf extracts of the two vegetables showed inverse relationship between the

increase in concentrations and the *in vitro* antioxidant activities with the ethanol leaf extract of *O. canum* having higher activity than the *V. amygdalina* at low concentrations and vice versa at higher concentrations as seen in Figure 2. However, ascorbic acid had more DPPH-scavenging (*in vitro antioxidant*) potential than the leaf extracts of the plants at the concentrations considered in this work (Figure 1 and 2).



**Figure 2.** Comparing the free radical, (DPPH), scavenging activity of ethanol extracts of *V. amygdalina* and *O. canum* using ascorbic acid as a positive control. Values are means of triplicate results of the inhibition.

**Table 2.** The effect of pretreatment with 400gm/kg of body weight aqueous leaf extracts of *V. amygdalina* and *O. canum* on the serum activities of AST, ALT and ALP of rats administered with 2 g/kg of body weight single dose of acetaminophen.

Treatment	AST (u/l)	ALT (u/l)	ALP (u/l)
Untreated group	41.90±3.30	27.90±8.20	86.30±58.80
Standard control	42.80±10.80	17.90±3.10	167.30±107.70
Negative control	67.40±26.40 <sup>++</sup>	117.30±57.50 <sup>++</sup>	209.80±67.00 <sup>++</sup>
<i>Vernonia amygdalina</i>	118.00±13.00*	9.05±3.76 <sup>**</sup>	104.00±56.00 <sup>**</sup>
<i>Ocimum canum</i>	147.00±4.48*	66.33±14.58 <sup>**</sup>	201.00±51.88

Values are mean ± SD of six (6) results, \* and \*\* show values with significant increase and decrease respectively, compared to the Negative control while <sup>++</sup> and <sup>^^</sup> indicate values with significant increase and decreases respectively compared to the Untreated group.

2 g per kilogram body weight (2g/kg of body weight), single dose acetaminophen caused significant rise in activities of ALT, AST and ALP of the animals administered with the drug without pretreatment with either the extracts or the standard hepatoprotective drug (Silymarin) when compared to the untreated group. Pretreatment with the extracts significantly reduced the

activities of ALT and ALP in the serum of the rats. The activity of AST in the serum was however, raised significantly in the extract pretreated rats (Table 2 and 3). There was significant ( $p < 0.05$ ) decrease in the concentration of albumin of the animals administered with a single 2g/kg of body weight of acetaminophen without pretreatment with either the extracts or the standard

**Table 3.** The effect of pretreatment with 400 gm/kg of body weight ethanol leaf extracts of *V. amygdalina* and *O. canum* on the serum activities of AST, ALT and ALP of rats administered with 2g/kg of body weight single dose of acetaminophen.

Treatment	AST (u/l)	ALT (u/l)	ALP (u/l)
Untreated group	41.90±3.30	27.90±8.20	86.30±58.80
Standard control	42.80±10.80	17.90±3.10	167.30±107.70
Negative control	67.40±26.40 <sup>++</sup>	117.30±57.50 <sup>++</sup>	209.80±67.00 <sup>++</sup>
<i>Vernonia amygdalina</i>	74.73±11.97	29.27±13.59 <sup>**</sup>	104.67±25.01 <sup>**</sup>
<i>Ocimum canum</i>	142.60±51.81 <sup>*</sup>	57.70±25.08 <sup>**</sup>	129.00±25.36

Values are mean ± SD of six (6) results, \* and \*\* show values with significant increase and decrease respectively, compared to the Negative control while <sup>++</sup> and <sup>---</sup> indicate values with significant increase and decreases respectively compared to the untreated group.

**Table 4.** The effect of pretreatment with 400 gm/kg of body weight aqueous leaf extracts of *V. amygdalina* and *O. canum* on the serum concentrations of total protein, albumin, and bilirubin of rats administered with 2g/kg of body weight single dose of acetaminophen.

Treatment	Total protein (g/dl)	Albumin (g/dL)	Total Bilirubin(mg/dl)	Direct Bilirubin(mg/dl)	Indirect. Bilirubin(mg/dl)
Untreated group	75.10±5.70	18.67±5.00	12.50±2.70	5.30±1.40	5.90±0.70
Standard control	76.80±10.50	4.70±2.30	9.00±5.20	6.50±2.90	4.00±3.30
Negative control	62.80±1.20	4.00±1.42 <sup>---</sup>	16.40±4.20	4.30±2.50	7.20±1.90
<i>V. amygdalina</i>	61.16±11.94	6.00±2.00	11.80±2.00	2.65±0.75	9.15±2.75
<i>O. canum</i>	77.57±12.48	10.67±2.31 <sup>*</sup>	8.87±1.96	1.20±0.35	4.67±0.83

Values are mean ± SD of six (6) results, \* and \*\* show values with significant increase and decrease respectively, compared to the Negative control while <sup>++</sup> and <sup>---</sup> indicate values with significant increase and decreases respectively compared to the Untreated group.

**Table 5.** The effect of pretreatment with 400mg/kg of body weight ethanol leaf extracts of *Vernonia amygdalina* and *Ocimum canum* on the serum concentrations of total protein, albumin, and bilirubin of rats administered with 2g/kg of body weight single dose of acetaminophen.

Treatment	Total protein (g/dl)	Albumin (g/dL)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Indirect. bilirubin (mg/dl)
Untreated group	75.10±5.70	18.67±5.00	12.50±2.70	5.30±1.40	5.90±0.70
Standard control	76.80±10.50	4.70±2.30	9.00±5.20	6.50±2.90	4.00±3.30
Negative control	62.80±1.20	4.00±1.42 <sup>---</sup>	16.40±4.20	4.30±2.50	7.20±1.90
<i>V. amygdalina</i>	78.48±11.29	12.00±6.93 <sup>*</sup>	18.43±5.40	6.13±4.52	8.97±6.81
<i>O. canum</i>	75.00±2.65	13.33±2.31 <sup>*</sup>	3.63±1.85 <sup>**</sup>	2.33±1.60	0.83±0.58 <sup>**</sup>

Values are mean ± SD of six (6) results, \* and \*\* show values with significant increase and decrease respectively, compared to the negative control while <sup>++</sup> and <sup>---</sup> indicate values with significant increase and decreases respectively compared to the untreated group.

hepatoprotective drug (Silymarin) when compared to the untreated group. Pretreatment with the extracts raised the serum concentrations of the rats as seen in Tables 4 and 5.

2 g per kilogram bodyweight, (2g/kgbw) of acetaminophen reduced significantly the activities of SOD and GPx when compared to the negative control (Tables 6 and 7). The pretreatment of the rats with 400 mg/kg of body weight of the aqueous and ethanol leaf extracts of *V. amygdalina* and *O. canum* all led to significant increase in the activities of these enzymes (SOD and GPx).

2 g per kilogram body weight (2g/kg of body weight), single dose acetaminophen caused the perturbation of the liver as evidenced in the significantly raised activities of ALT, AST and ALP in the negative control as compared to the untreated group. The liver injury suggested here is in consonance with the work of Prabu *et al.*, 2011 and Ekor *et al.*, 2006, which reported liver damage as a result of the administration of 2g/kg of body weight of acetaminophen in albino rats. The activities of the liver antioxidant enzymes, SOD and GPx were significantly reduced in the homogenate of the negative control, compared to the untreated group. The activity of

**Table 6.** The effect of pretreatment with 400mg/kg of body weight of aqueous leaf extracts of *V. amygdalina* and *O. canum* on the activities of the liver antioxidant enzymes of rats administered with a 2g/kg of body weight single dose of acetaminophen.

Samples	SOD (U/mg)	CAT (U/mg)	GPx ( $\mu$ /mg)
Untreated group	0.05 $\pm$ 0.01	3.00 $\pm$ 2.30	397.00 $\pm$ 100.00
Standard Control	0.20 $\pm$ 0.40	20.90 $\pm$ 14.90	184.60 $\pm$ 23.60
Negative Control	0.02 $\pm$ 0.01 <sup>~</sup>	3.00 $\pm$ 2.60	115.60 $\pm$ 10.03 <sup>~</sup>
<i>V. amygdalina</i>	0.04 $\pm$ 0.03*	13.56 $\pm$ 11.42*	329.41 $\pm$ 87.25*
<i>O. canum</i>	0.01 $\pm$ 0.00**	2.74 $\pm$ 1.87	233.49 $\pm$ 30.00*

Values are mean  $\pm$  SD of six (6) results, \* and \*\* show values with significant increase and decrease respectively, compared to the Negative control while <sup>~</sup> and <sup>~</sup> indicate values with significant increase and decreases respectively compared to the untreated group.

**Table 7.** The effect of pretreatment with 400mg/kg of body weight of aqueous leaf extracts of *V. amygdalina* and *O. canum* on the activities of the liver antioxidant enzymes of rats administered with a 2g/kg of body weight single dose of acetaminophen.

Samples	SOD (U/mg)	CAT (U/mg)	GPx ( $\mu$ /mg)
Untreated group	0.05 $\pm$ 0.01	3.00 $\pm$ 2.30	397.00 $\pm$ 100.00
Standard Control	0.20 $\pm$ 0.40	20.90 $\pm$ 14.90	184.60 $\pm$ 23.60
Negative Control	0.02 $\pm$ 0.01 <sup>~</sup>	3.00 $\pm$ 2.60	115.60 $\pm$ 10.03 <sup>~</sup>
<i>V. amygdalina</i>	0.03 $\pm$ 0.01*	11.25 $\pm$ 4.86*	249.12 $\pm$ 98.50*
<i>O. canum</i>	0.09 $\pm$ 0.06*	6.54 $\pm$ 1.95*	203.83 $\pm$ 63.93*

Values are mean  $\pm$  SD of six (6) results, \* and \*\* show values with significant increase and decrease respectively, compared to the Negative control while <sup>~</sup> and <sup>~</sup> indicate values with significant increase and decreases respectively compared to the untreated group.

CAT was also reduced in the negative control, although the reduction was not statistically found to be significant when compared to the untreated group. This is an indication of the induction of oxidative stress in the liver. Disrupted hepatic lobular architecture of the rats was also observed, all these alterations were seen in the negative control (group 3) as compared to the untreated group (group 1).

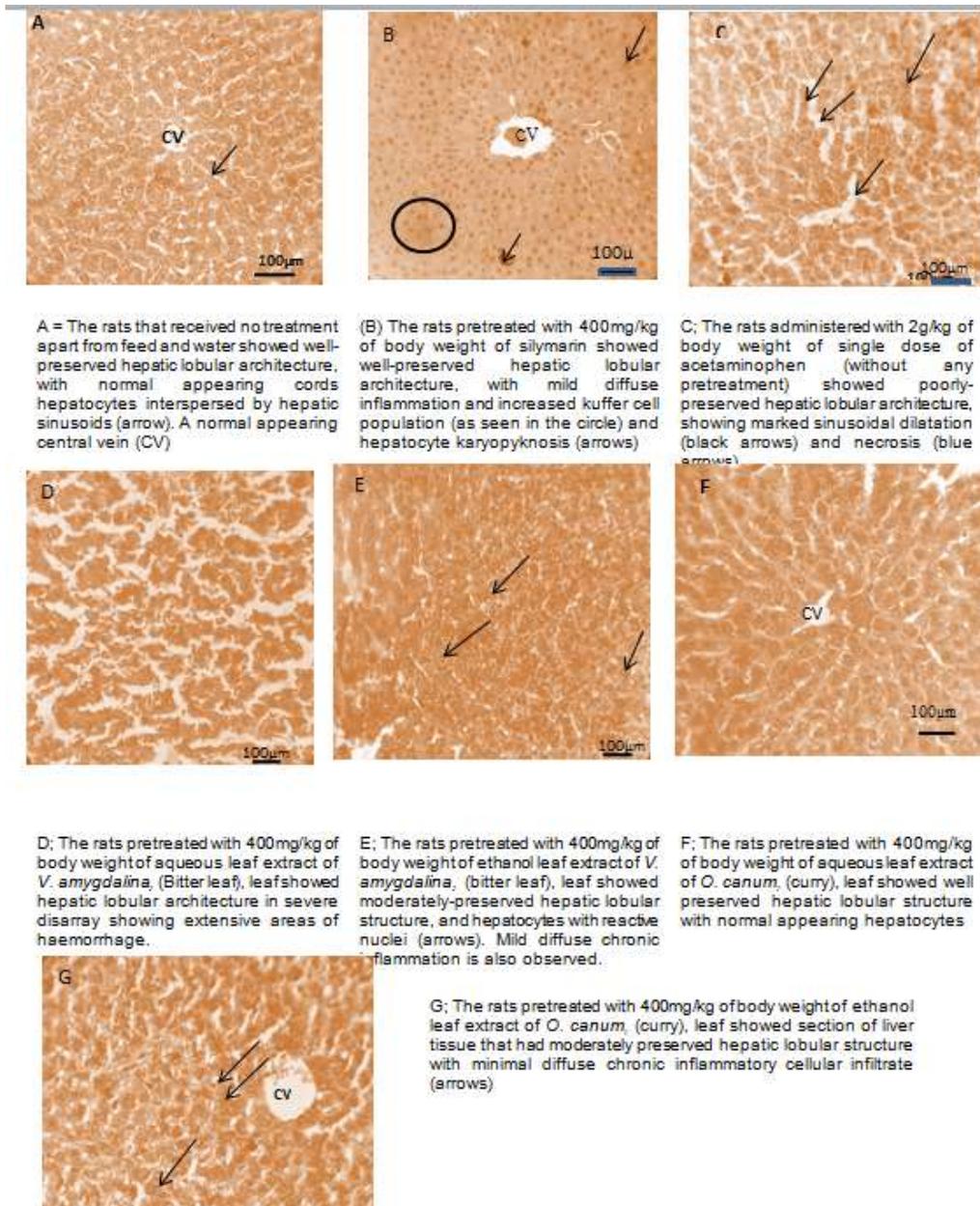
Acetaminophen also altered the concentration of protein (total protein and albumin) in the serum of the rats, which could be as a result of the binding of NAPQI to proteins (Mitchell *et al.*, 1973) or the effect of NAPQI on the protein synthesizing/metabolizing ability of the liver. The aminotransferase are abundant in the liver and are released into the bloodstream following hepatocellular damage, making them sensitive markers of liver damage (Al-Mamary, 2002; Sarvesh, 2012).

Therefore, marked increases in the serum ALT and AST activities reported in the present study is an indication of liver damage. Serum activity levels of aminotransferases is used as an indicator of damage to the liver structural integrity because these enzymes are cytoplasmic in location and are released into the

circulating blood only after structural damage. (Okediran *et al.*, 2014). A marked rise in the serum activity of ALT, reduction in total serum protein and abnormal increase in serum bilirubin had been reported in hepatotoxicity, (Olamide and Mathew, 2013; Olorunnisola *et al.*, 2011; Martin and Friedman, 1992).

*N*-acetyl-*p*-benzoquinone imine (NAPQI), is an oxidative product of acetaminophen metabolism that binds covalently to the sulfhydryl groups of proteins resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver causing hepatotoxicity (Kanchana and Mohammed, 2011). Results from the present study provides evidence of the induction of oxidative stress nine hours (9 h), following 2g/kg of body weight acetaminophen (APAP), administration.

The induced oxidative stress as found in the study is evident in the significantly decreased activities of the SOD, CAT and the GPx of the animals in the negative control group as compared to the untreated group, resulting in decrease in cellular defence against oxidative damage. The increased formation of superoxide may have led to hydrogen peroxide formation as found in James *et al.* (2003).



**Figure 3.** The effect of pretreatment with 400mg/kg of body weight of leaf extracts of *V. amygdalina* and *O. canum* on the histopathology of livers of rats administered with a 2g/kg of body weight single dose of acetaminophen

Ekor et al. (2006) reported that after 7 h, following paracetamol (PCM) administration, there was a rise in GST activity, indicating increased GST-catalysed conjugation of NAPQI with GST that led to the depletion of cellular GSH level. Histological profile of the livers of the rats in the negative control group showed a poorly preserved hepatic lobular architecture, sharply demarcated hepatocyte, necrosis and exhibiting periportal sinusoidal congestion, which is a confirmation of liver injury.

Previous studies by Densie (2013), Prerona et al. (2011), Huang et al. (1992), Ekor et al. (2006), Kanchana and Mohammed (2011) and Okediran et al. (2014) had all shown extracts from plant to be potent in hepatoprotection. The present study provides evidence that the pretreatment of rats, with a 400mg/kg of body weight per day with aqueous or ethanol leaf extract of *V. amygdalina* and *O. canum* for seven days, was hepatoprotective against toxicity and oxidative stress arising from a 2g/kg of body weight oral administration

with acetaminophen over nine hours. The pretreatment with the leaf extract led to significant decrease of the serum activities of ALT and ALP of the animals in the test groups when compared to that of those in the negative control. While Silymarin gave 85% decrease, the aqueous leaf extracts of *V. amygdalina* and *O. canum*, gave 92 and 43% decrease respectively in the activity of ALT in the serum of the rats as compared to the negative control. ALP activity in the serum was decreased by 70, 50 and 4.3% as a result of the pretreatment with silymarin, aqueous leaf extracts of *V. amygdalina* and *O. canum* respectively.

The ethanol leaf extracts of the plants (*V. amygdalina* and *O. canum*) decreased the serum ALT of the rats by 75 and 50%, respectively. While the ethanol leaf extract of *V. amygdalina* and *O. canum* led to 50 and 39% decrease in ALP activity, respectively. This suggests that the leaf extracts of the plants are hepatoprotective. The aqueous leaf extract of *V. amygdalina* offered the best protection, followed by Silymarin, ethanol leaf extract of *V. amygdalina*, ethanol leaf extract of *O. canum* and aqueous leaf extract of *O. canum* in that order. The binding of NAPQI to proteins (Kanchana and Mohammed, 2011), may have been inhibited by pretreatment with silymarin, aqueous and ethanol leaf extracts of *V. amygdalina* and *O. canum* in this study. The increase in albumin concentration in the serum was in the increasing order of silymarin (18%) < aqueous leaf extract of *O. canum* (167%) < ethanol leaf extract of *V. amygdalina* (200%) < ethanol leaf extract of *O. canum* (233%) < aqueous leaf extract of *V. amygdalina* (267%).

The activities of CAT, SOD and GPx were increased significantly at 95% confidence level by the actions of the leaf extracts. Catalase, Superoxide Dismutase and Glutathione Peroxidase are the primary intracellular defence mechanism to cope with increased oxidative stress, eliminating superoxide anion and hydrogen peroxide that may oxidise cellular substrates thereby preventing free radical chain reactions (Ekor et al., 2006).

The induction of higher activities of these antioxidant enzymes is a suggestion of the protection of the livers by reducing oxidative stress on the liver. The Silymarin showed the highest increase of CAT activity, while aqueous leaf extract of *V. amygdalina* led to the highest increase in GPx activity and ethanol leaf extract of *O. canum* gave the highest (350%) increase in SOD activity, compared to the negative control. These hepatoprotective potentials were also corroborated by the histopathological studies. All these protections may be due to the antioxidant properties of the plants, which stem from their phytochemical components. It has been found that phytochemicals have the potential to reduce oxidative damage to cells (Densie, 2013, Prerona et al., 2011; Huang et al., 1992).

In this study, the aqueous leaf extract of *V. amygdalina* gave the best hepatoprotective effect, even better than the standard drug, followed by the ethanol extract of the

same plant material. This could stem from the fact that the aqueous and ethanol leaf extract of *V. amygdalina* contains phenols that were not detected in the aqueous leaf extract of *O. canum*. The better activity of the ethanol leaf extract over the aqueous leaf extract of *O. canum* can also be attributed to the presence of phenols in the ethanol extract.

## Conclusion

The leaves of the plants were found to be potential antioxidants and hepatoprotective (and the compared well with the standard drug Silymarin), with the best result obtained from the aqueous leaf extract of *V. amygdalina*. Therefore, they can be good sources of raw materials for the production of drugs for the prevention and treatment of liver and associated diseases.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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