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Comparative Analysis of Phytochemical and Antimicrobial effects of Extracts of some Local Herbs on Selected Pathogenic Organisms[1]

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Abstract – This study principally focused on three medicinal plants (*Vernonia amygdalina, Gongronema latifolium* and *Ocimum basilicu*m) for their phytochemical and antimicrobial qualities against some microorganisms. The samples were collected locally from Nekede in Owerri West Local Government Area of Imo State, Nigeria and analyzed using standard microbiological procedures. The phytochemical compositions were evaluated using direct chemical composition and thin layer chromatography. The result showed that the leaf extracts of *Vernonia amygdalina, Gongronema latifolium* and *Ocimum basilicum* possess the phytochemicals: tannins, saponins, alkaloids, flavonoids, cardiac glycosides, cynogenic glycosides. The active components of the leaf samples were extracted using solvent extraction technique. The solvents used are acetone and ethanol. The method described by Akerele et al., 2008) was used to extract the bioactive components from the powdered samples. The methods of Akerele et al.,(2008) was slightly modified. The modification was based on Doughari and Manzara (2008). The antimicrobial efficacies of the extracts were tested against *Klebsiella pnuemonea, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* using disc diffusion method. The Minimum Inhibitory Concentration of each was also ascertained. The result showed that acetone and ethanol extracts of Vernonia amygdalina had highest zones of inhibition on *Klebsiella pnuemonea, Pseudomonas aeruginosa* was intermediate. *E .coli* and *Staph aureus* were resistant to the low concentrations used. Acetone and ethanolic extracts of *Ocimum basilicum* had highest inhibition on *Pseudomonas aeruginosa*, intermediate on *Klebsiella pnuemonea* and *Staphylococcus aureus* and with highest on *E.coli. Acetone* and ethanolic extracts of *Gongronema latifolium* showed that *Klebsiella pnuemonea, E. coli, Staphylococcus aureus* were sensitive to it but *Pseudomonas aeruginosa* was intermediate to it. Therefore the study suggests the possible exploration of these plants as sources of natural product for future use in the management of multi-drug resistant pathogens such as *E. coli, Klebsiella pnuemonea, Staphylococcus aureus* and *P. aeruginosa* that cause wide range of infections.

Keywords – *phytochemicals, antimicrobial, Vernonia amygdalina, Gongronema latifolium, Ocimum basilicum,pathogenic organisms.*

1. Introduction

1.1. *Vernonia amygdalina*

Vernonia amygdalina is a valuable medicinal plant that is widespread in East and West Africa (Burkill, 1985). It is known as bitter leaf, due to its characteristics bitter taste and flavor, and may be used as an active anticancer (Izevbigie, 2003),antibacterial, antimalaria agent (Tadesse et al., 1993). This plant contains complex active components that are pharmacologically useful. The bitterness is caused by sesquiterpene lactones (e.g. vernodalin, vernolepin and vernomygdin) and steroid glucosides (vernoniosides). Some of these compounds have significant antiparasitic activity, especially vernodalin and vernonioside B1 (Calixto, 2000; Smith, 2008; Finar, 2008). Vernolepin showed platelet anti-aggregating properties. Vernodalin and vernomygdin have cytotoxicactivity (Calixto, 2000).

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1.2. *Ocimum basilicum*

Ocimum basilicum has been reported to be active against several species of bacteria and fungi (Nwosu, 1995; Nakaruma et al., 1999). It is known as sweet basil. The various basils have such different scents because the herb has a number of different essential oils that come together in different proportions for various breeds. The strong clove scent of sweet basil is derived from eugenol, the same chemical as actual cloves (Pessoa et al., 2002). African blue basil has a strong camphor smell because it contains camphor and camphene in higher proportions (Bozin et al., 2006).

1.3. *Gongronema latifolium*

Gongronema latifolium, commonly called "utazi" and "arokeke" in the South Western and South Eastern parts of Nigeria, is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine (Ugochukwu and Babady, 2002; Ugochukwu et al., 2003). Reports by various authors showed that it contains essential oils, saponins and pregnanes among others (Schneider et al., 1993; Morebise and Fafunso, 1998; Morebise et al., 2002).Ugochukwu et al., (2003) and Ogundipe et al., (2003) reported that aqueous and ethanolic *G. latifolium* extracts had hypoglycemic, hypolipidemic and antioxidative properties while Morebise et al., (2002) showed that it has anti-inflammatory properties. *Gongronema latifolium* is widespread in tropical Africa and occurs in the triangle between Senegal in the West to Chad in the West and Congo in the South. It has widespread use for medicinal and nutritional purposes. An infusion of the aerial parts is taken to treat cough, intestinal worms, dysentery, and malaria. It is also taken as a tonic to treat loss of appetite. In Sierra Leone an infusion or decoction of the stems with lime juice is taken as a purge to treat colic and stomachache. In Senegal and Ghana the leaves are rubbed on the joints of small children to help them walk. The boiled fruits in soup are eaten as a laxative (Akuodor et al., 2010). In Nigeria a leafy stem infusion is taken as a cleansing purge by muslims during Ramadan. A decoction of leaves or leafy stems is commonly taken to treat diabetes and high blood pressure. The latex is applied to teeth affected by caries. It is also taken for controlling weight gain in lactating women and overall health management. Asthma patients chew fresh leaves to relieve wheezing. A cold maceration of the roots is also taken as a remedy for asthma. A decoction of the roots, combined with other plant species, is taken to treat sickle cell anemia. A maceration of the leaves in alcohol is taken to treat bilharziosis, viral hepatitis and as a general antimicrobial agent 1. Few chemical analyses have been performed on *Gongronema latifolium.* From the leaves several 17β-marsdenin derivatives (pregnane glycosides) were isolated, as well as β-sitosterol, lupenyl cinnamate, lupenyl acetate, lupeol, essential oils and saponins. The essential oil from the leaves contains as main components linalool (19.5%), (E)-phytol (15.3%) and aromadendrene hydrate (9.8%)4,5,6

2. Materials and method

Media: Nutrient agar, Mueller Hinton agar, nutrient broth and peptone water.

Extract: Ethanolic and Acetonic extracts of *Ocimum basilicum* (sweet basil); Ethanolic and Acetonic extracts of *Vernonia amygdalina* (bitter leaf); Ethanolic and Acetonic extracts of *Gongronema latifolium* (utazi leaf).

2.1. *Processing of the plant leaves*

The leaves were processed according to the method of Atata et al., (2003). The leaves were collected by hand plucking from plant and cleaned of debris. The leaves were then air-dried at room temperature for 14 days. The dried leaves were blended using a manual grinder. Powdered samples were stored in tightly closed reagent bottles for subsequent extraction and bioassay.

2.2. *Preparation of extracts*

The method described by Akerele et al., (2008) was used to extract the bioactive components from the powdered samples. The methods of Akerele et al., (2008) was slightly modified. The modification was based on Doughari and Manzara (2008). To 10g portion of the leaf powder, one hundred milliliters (100ml) each of ethanol, and acetone were added in separate sterile conical flasks and allowed to soak at room temperature for 24 hours. The samples were periodically shaken for at least 2 hours a day using an electric shaker to ensure complete extraction (Nenaah and Ahmed, 2011). The extracts were then filtered using filter paper (Whatman No 1), and the filtrates concentrated at 400C using a rotary evaporator (Akerele et al., 2008; Nenaah and Ahmed, 2011). The residues obtained were dried and stored at 40C until bioassayed.

2.3. *Sterility test of the extract*

The acetonic and ethanolic extracts of the plants were tested for sterility using the method of Dalitha (2008). 1ml of each extracts was added into test tube containing 5ml of sterile nutrient broth. They were then incubated at 370C for 24hrs. The extracts were clear after incubation indicating the absence of contaminant which would have caused a turbid appearance in the tubes.

2.4. *Collection and confirmation of test organisms*

The bacterial isolates include: *Escharichia. coli, Staphylococcus aureus, klebsiella pnuemonea* and *Pseudomonas aeuroginosa.* The isolates were obtained from the Federal Medical Centre (FMC), Owerri, Imo State. The collected isolates were subjected to cultural, morphological and biochemical characteristics as described by Cheesbrough, (Cheesbrough, 2000) and compared with criteria in the Bergey's Manual of Determinative Bacteriology (1993).

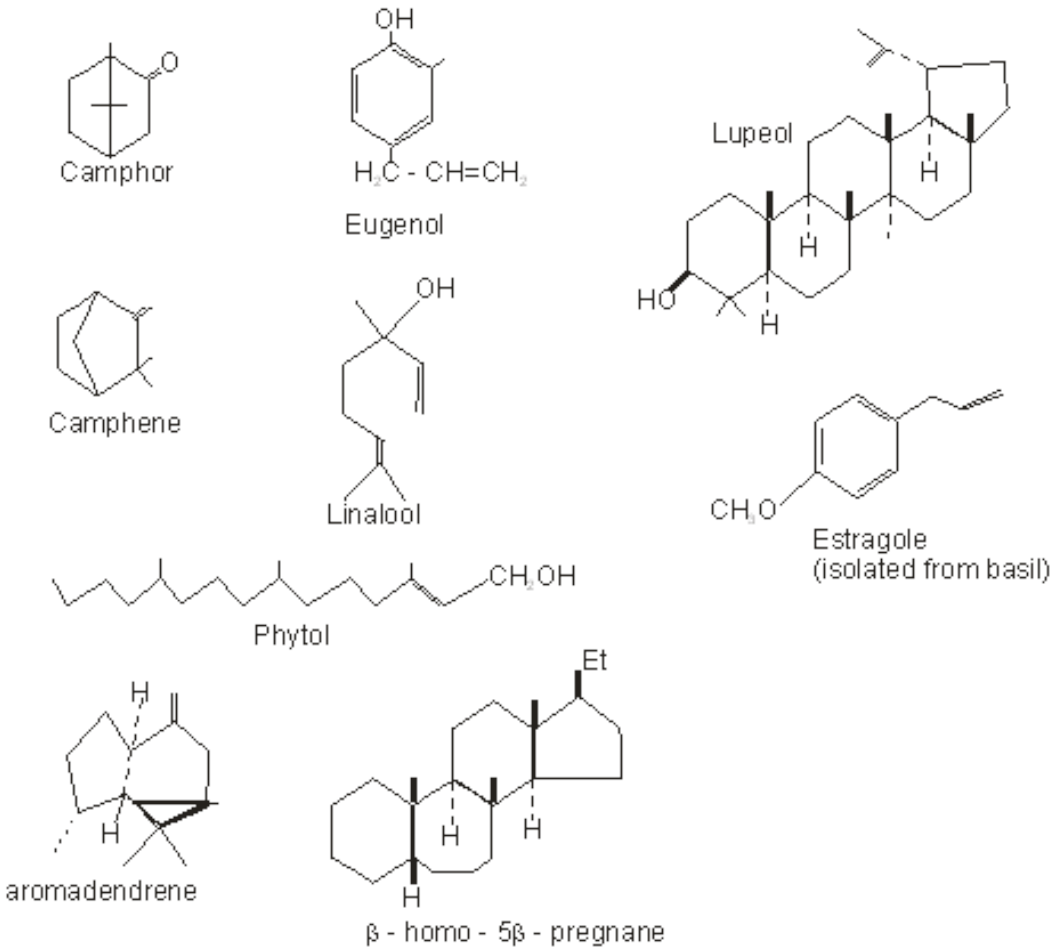

Figure 1: Representations of the molecules being used in the study

2.5. Determination of phytochemical constituents

Phytochemical quantitative analyses were conducted on the acetonic and ethanolic extracts of each plant as described by Amadi et al., (2004). The leaves were screened for tannins, alkaloids, flavonoids, saponins, cynogenic and cardiac glycosides. Qualitatively by specific solvent system thin layer chromatography using precoated silica gel in Shandon Unikit TLC Chromatank.

2.6. Antimicrobial activity bioassay

The extracts were screened for antimicrobial activity using the disc diffusion method as described by Dalitha (2008).

2.7. Standardization of inocula

The cultures were standardized using the method of Dalitha (2008). Pure isolates were transferred into sterile prepared nutrient broth and the cultures were then adjusted to 0.5 McFarland turbidity standards by checking the turbidity level in comparison with the standard.

2.8. Determination of minimum inhibitory concentration (MIC) of extracts

Determination of the minimum inhibitory concentration (MIC) was carried out using the broth dilution method as described by Dalitha (2008).The MIC of the extracts were determined for the test organisms in triplicates at varying concentrations. A stock solution of 20mg/10ml was prepared for each extract and ciprofloxacin separately. One milliliter (1ml) of nutrient broth was dispensed into test tubes and sterilized by autoclaving at 1210C at 15psi for 15min. The different extracts and ciprofloxacin were serially diluted from their stock solutions to obtain varying concentrations. The concentrations were: 1.0, 0.5 and 0.25 mg/ml. 0.1ml of each test isolate was inoculated into the various test tubes containing varying concentrations and then, incubated at 370C for 24h. After incubation, the presence or absence of growth on each tube was rated.

3. Result

Table 1 on page 244 is the zones of inhibition of the leave extracts. The acetone and ethanol extracts of V. *amygdalina* exhibited highest zones of inhibition on K. *pnuemonea* 20mm and 18mm respectively, followed by P.*aeruginosa* 7mm. The two extracts have no effect on both *S. aureus* and *E. coli*. The two extracts of *Ocimum*

Table 1: Zone of inhibition of the leave extracts on isolates.

Test Isolates	Vernonia amygdalina		Ocimum basilicum		Gongronema latifolium	
	Acetone	Ethanol	Acetone	Ethanol	Acetone	Ethanol
Klebsiella pnuemonea	20	18	9	7	10	14
Escherichia coli	0	0	13	12	12	12
Staphylococcus aureus	0	0	9	9	11	10
Pseudomonas aeruginosa	7	7	11	8	8	7

basilicum inhibited all the test organisms. The acetone and ethanol extracts of *Ocimum basilicum* had the highest zones of inhibition on *E. coli* 13mm and 12mm respectively. Also the two extracts of *Gongronema latifolium* inhibited all the test organisms with the highest zone shown by the ethanolic extract on K. *pnuemonea* 14mm.

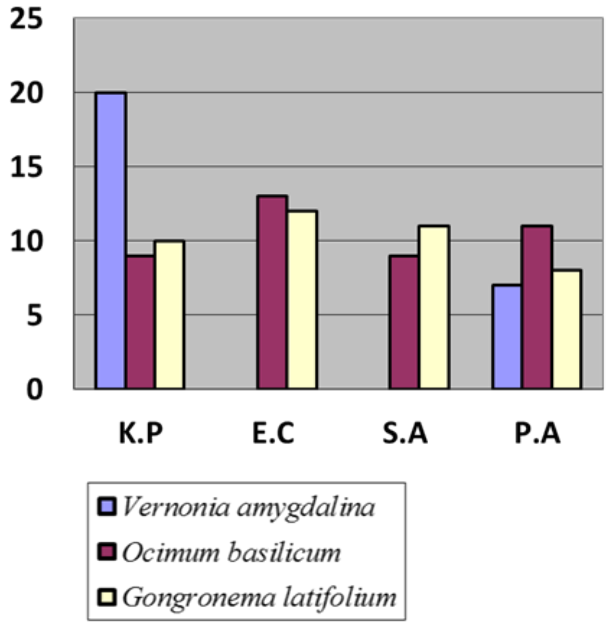

Figure 2: Zones of inhibition of acetone extract on test organisms. (K.P = Klebsiella pneumonia; E.C = Escharichia coli; S.A = Staphylococcus aureus; P.A = Pseudomonas aeruginosa

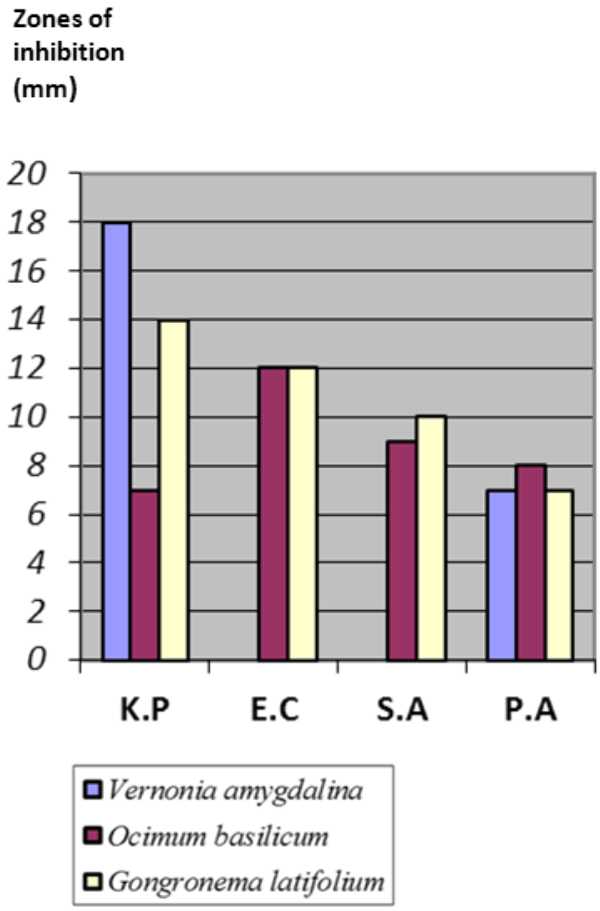

Figure 3: Zones of inhibition of ethanol extract on test organisms

In comparism, the acetonic extract of the three plants showed that V. amygdalina exhibited the highest zone of inhibition 20mm, followed by *Gongronema latifolium* 10mm, then *Ocimum basilicum* 9mm on *Klebsiella pnuemonea*. *Ocimum basilicum* gave the highest zone of inhibition on *E. coli* 13mm than *Gongronema latifolium* 12mm. *V. amygdalina* has no antimicrobial activity on *E.coli*. *Gongronema latifolium* has the highest antimicrobial activity on *S. aureus* 11mm, followed by *Ocimum basilicum* 9mm. S. aureus was not inhibited by *V. amygdalina*. *P.aeruginosa* was inhibited most by *Ocimum basilicum* 11mm next by *Gongronema latifolium* 8mm, and finally by *V. amygdalina* 7mm.

The ethanolic extract of the three plants showed that V. amygdalina exhibited the highest zone of inhibition 18mm, followed by *Gongronema latifolium* 14mm, then *Ocimum basilicum* 7mm on *Klebsiella pnuemonea*. *Ocimum basilicum* and *Gongronema latifolium* gave the highest zone of inhibition on E. coli 12mm, while *V. amygdalina* has no antimicrobial activity on *E. coli*. *Gongronema latifolium* has the highest antimicrobial activity on *S. aureus* 10mm, followed by *Ocimum basilicum* 9mm. *S. aureus* was not inhibited by *V. amygdalina*. *P.aeruginosa* was inhibited most by *Ocimum basilicum* 8mm, followed by both *Gongronema latifolium* and *V. amygdalina* 7mm.

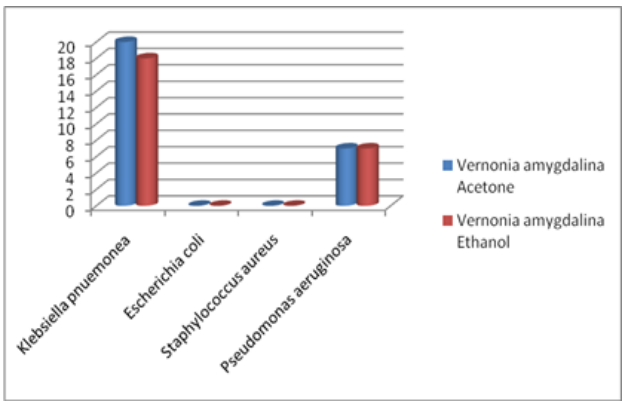

Figure 4: Zone of inhibition of both acetone and ethanol extracts of ***Vernonia amygdalina*** against test isolates. The acetonic extract of *V. amygdalina* gave the highest zone of inhibition on Klebsiella pneumonia 20mm than the ethanolic extract which gave 18mm. Both *E. coli* and *S. aureus* were not inhibited by the two extracts. The two extracts gave equal zones of inhibition 7mm on Pseudomonas aeruginosa

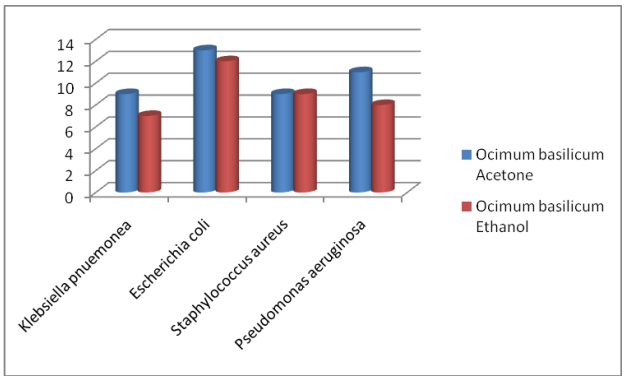

Figure 5: Zone of inhibition of both acetone and ethanol ***extracts of Ocimum basilicum*** against test isolates.

The two extracts of Ocimum basilicum showed varied zones of inhibition on all the test organims. The acetonic extract exhibited a higher zone of inhibition 9mm on Klebsiella pneumonia as compared to ethanolic extract that gave a zone of inhibition of 7mm. E. coli was inhibited more by acetonic extract 13mm than the ethanolic extract which gave a zone of inhibition of 12mm. The acetonic and ethanolic extracts of Ocimum basilicum showed equal zones of inhibition 9mm on S. aureus. The acetonic extract of Ocimum basilicum gave a higher zone of inhibition 11mm on Pseudomonas aeruginosa than the ethanolic extract which gave 8mm.

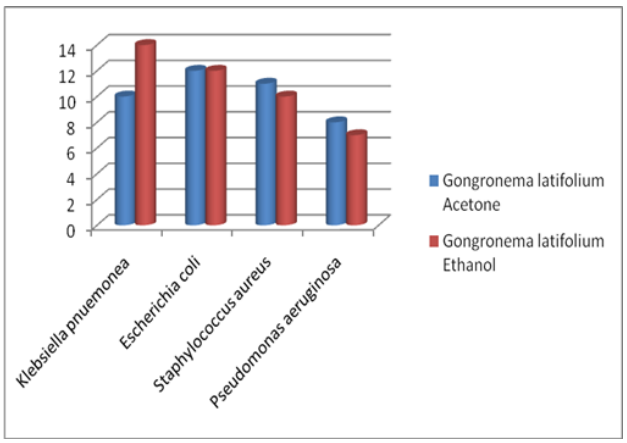

Figure 6: Zone of inhibition of both acetone and ethanol extracts of G***ongronema latifolium*** against test isolates.

The two extracts of Gongronema latifolium showed varied zones of inhibition on all the test organims. The ethanolic extract exhibited the highest zone of inhibition 14mm on Klebsiella pneumonia than the ethanolic extract that gave a zone of inhibition of 10mm. The acetonic and ethanolic extracts of Ocimum basilicum showed equal zones of inhibition 12mm on E. coli. S. aureus was inhibited more by acetonic extract 11mm than the ethanolic extract which gave a zone of inhibition of 10mm. The acetonic extract of Ocimum basilicum gave a higher zone of inhibition 8mm on Pseudomonas aeruginosa than the ethanolic extract which gave 7mm.

The minimum inhibitory concentration of the extracts on the test organism is shown on Table 2 on page 246. The lowest concentration 0.25 of both extracts of the three leaves used did not inhibit the growth of all the test organisms. The acetonic extract of Vernonia amygdalina 0.5conc inhibited the growth of only Klebsiella pneumonia, the 1.0 conc inhibited the growth of only Klebsiella pneumonia and Pseudomonas aeruginosa. The ethanolic extract of Vernonia amygdalina 0.5conc inhibited the growth of only Klebsiella pneumonia while the 1.0 conc inhibited the growth of Klebsiella pneumonia and Pseudomonas aeruginosa. The 1.0 conc of both the acetonic and ethanolic extracts of Ocimum basilicum suppressed the growth of all the test organisms. The 0.5conc of the acetonic extract inhibited the growth of only E.coli while the 0.5 conc of the ethanolic extract suppressed the growths of E. coli and S. aureus. The 1.0 conc of both the acetonic and ethanolic extracts of Gongronema latifolium suppressed the growth of all the test organisms. The 0.5conc of the acetonic extract inhibited the growth of only S. aureus while the 0.5 conc of the ethanolic extract suppressed the growths of Klebsiella pneumonia and S. aureus.

Table 2: Minimum Inhibitory Concentration of the acetonic and ethanolic extract.

EXTRACTS	CONCENTRATION OF EXTRACTS (mg/ml)	Klebsiella pneumonia	Escherichia coli	Staphylococcus aureus	Pseudomonas aeruginosa
Vernonia amygdalina	A. 1.0	-	++	++	-
	0.5	-	++	++	++
	0.25	++	+++	+++	+++
	E. 1.0	-	+	+	-
	0.5	-	++	++	++
	0.25	++	++	++	++
Ocimum basilicum	A. 1.0	-	-	-	-
	0.5	+	-	+	++
	0.25	++	+	++	+++
	E. 1.0	-	-	-	-
	0.5	++	-	-	+
	0.25	++	++	+	++
Gongronema latifolium	A. 1.0	-	-	-	-
	0.5	+	++	-	+
	0.25	++	++	++	++
	E. 1.0	-	-	-	-
	0.5	-	++	-	++
	0.25	++	++	+	++

- No growth
+ Scanty growth
++ Moderate growth
+++ Heavy growth
A Acetone
E Ethanol

Table 3: Phytochemical analysis of leaves

BIOACTIVE COMPONENTS	Vernonia amygdalina	Ocimum basilicum	Gongronema latifolium
Tannins	+	+	+
Saponins	+	+	+
Alkaloids	+	+	+
Flavonoids	-	+	+
Cardiac glycosides	+	+	+
Cynogenic glycosides	+	-	+

The phytochemical analysis of the leaves is shown on Table 3. The phytochemicals: tannins, saponins, alkaloids, flavonoids, cardiac glycosides and cynogenic glycosides were analysed and are present in the three leaves tested. Only cynogenic glycosides was absent in Ocimum basilicum.

4. Discussion

Antimicrobial activities of plants have been attributed to bioactive components, such as alkaloids, saponins, tannis, flavonoids, steroids, anthroquinones etc (Odugbemi, 2006). The work of Enyi-Idoh et al., (2012) also showed that Vernonia amygdalina and Gongronema latifolium have ample quantities of saponins, flavonoids, alkaloids and tannis. From this research it has been observed that the acetonic and ethanolic extracts of Ocimum basilicum and Gongronema latifolium can inhibit the growths of all the test organisms; but the acetone and ethanol extract of Vernonia amygdalina had highest zones of inhibition on Klebsiella pneumonia. Pseudomonas aeruginosa was intermediate while E. coli and Staphylococcus aureus were resistant to it. This resistance could be as a result of the lower concentrations of the extracts used (0.25, 0.5, 1.0 mg/ml), because in the studies of Ibrahim et al. (2009), the antimicrobial activity of the ethanolic extracts of V. amygdalina against S. aureus and E. coli were reported with MICs as high as 12.5mg/ml. Also Enyi-Idoh et al., (2012) reported that the highest activity of ethanolic extracts of both plants was at high MICs of 100mg/ml and above. The result of this research conforms with the works of (Eja et al., 2011; Oshodi et al., 2004) who reported the antimicrobial activities of Vernonia amygdalina and Gongronema latifolium on E. coli and Staphylococcus aureus.

5. Conclusion

Traditional plants may represent new sources of antimicrobial with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. This research shows the antimicrobial activity of V.amygdalina, O.bacilicum, G. latifolium using different solvent extractants acetone and ethanol on selected pathogens. However, local ethno medical prepa-

rations and prescriptions of the plant sources should be scientifically evaluated and disseminated properly. Furthermore, the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigations into the field of pharmacology, photochemistry, ethnobotany and other biological actions for drug recovery. This work suggests the possible exploration of these plants as sources of natural product for future use in the management of multi-drug resistant pathogens such as E. coli, Klebsiella pneumonia, Staphylococcus aureus and Pseudomonas aeruginosa that cause wide range of infections.

6. Recommendation

Pharmaceutical industries should also produce drugs from local herbs with less additives to retain the active ingredient in the leaves and also prevent diseases which could result from intake of drugs with excess additives.

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