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Chemical Characterization and Anti-Microbial Activity of Gongronema Latifolium (Utazi) Organic Extract

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ABSTRACT

E. coli, S. aureus, Bacillusspp and other bacteria cause great damage in food, beverage and pharmaceutical industries in West Africa. Illnesses caused by these organisms have also been on the rise in recent years, making it difficult for food storage as customers are looking elsewhere for solutions. The use of natural and herbal extracts as preservatives and medicines has been proven in recent times to be of greater advantage over the synthetic drugs and chemical preservatives. Locally, Gongronema latifolium has been used in the preservation of fruits and treatment of ailments. It is proposed that further research is needed in the use of Gongronema latifolium for medicinal and preservative purposes. This study is aimed at showing that organic extracts gotten fromG. latifolium is effective in growth inhibition and destruction of both gram positive and gram negative health deteriorating causing bacteria. This is done by extracting the organic matter from already prepared Gongronemalatifolium leaves using the Soxhlet apparatus and testing it on different five bacteria obtained from pure strains and cultured in the lab.Results gotten for the antimicrobial activity showed that the ethane extracts from Gongronema latifolium exhibited a maximum zone of inhibition (10 mm) against Staphylococcus aureus (MIC = 5 cfu/ml), while the ethyl acetate extracts showed the least zone of inhibition (1 mm) to Staphylococcus aureus (MIC = 0 cfu/ml). The methanolic extracts showed the maximum zone of inhibition (9.1 mm) to Escherichia coli (MIC = 4 cfu/ml) while the ethyl acetate extracts showed the least zone of inhibition (1 mm) to Escherichia coli (MIC = 0 cfu/ml). This result has proven the basis for this plant to be used particularly in the treatment of illness caused by microorganisms.

Key words: Antimicrobial Activity, Gongronema Latifolium, Organic Extracts, bacteria, staphylococcus.

1.INTRODUCTION.

Plant products have currently become of growing interest as antimicrobial agents of use in the future (Gundidza 1993). *Gongronema latifolium*, a plant product common in Nigeria; locally known as "utasi" by the Efiks, Ibibios and Quas, Igbos call it "utazi" and while the Yorubas call it "arokeke" or "madumaro". It is a herbaceous shrub, with yellow flowers that produces a characteristic milky latex when cut (Balogun *et. al.;* 2016; Itoro *et. al.*, 2015; Ugochukwu and Babady, 2002), belongs to a group of plants generally known as spices. It is famous in tropical Africa and can be found throughout Nigeria as well as from Senegal east to Chad and south to DR Congo (Etta *et. al.*, 2012; Owu *et. al.*, 2012). *Gongronema latifolium* is a climbing shrub with broad, heart-shaped leaves and has a characteristic sharp, bitter and slightly sweet taste, especially when eaten fresh (Omodale *et. al.*, 2017).

Abubakar *et. al.*, (2020), reported in their study that natural products of plant origin has overtime served and continued to serve as major sources of drugs against diseases such as cancer, diabetes and microbial infections amongst others. The use of plant based traditional medicine is widespread in Africa (Bading Taika *et. al.*, 2018) and particularly in developing countries as it has been in existence for several years. According to WHO (2001), a good percentage (80%) of the population of the world depends on traditional medicine which has compounds gotten from medicinal plant as a primary mode of disease treatment. Based on this statistics, there is a hike in rate among developing countries. It is considered very important to the wellbeing of humans (Elujoba *et. al.*, 2005; Okwu 2007).

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Gongronema latifolium is one of the most important plants and it is known for its nutritive and aromatic purposes and reports have shown that its extracts have antibacterial, antifungal, antioxidative, and antihelminthic properties (Odukoya *et. al.,* 2015; Bassey *et. al.,* 2020). The high nutritional content has been reported in various studies of *Gongronema latifolium* (Offor *et. al.,* 2015; Abu *et. al.,* 2014; Eleyinmi, 2007). According to Eleyinmi (2007), *Gongronema latifolium* has high phytochemical and protein content and the intake of 100 g (DM) of it may be capable of providing 27g of protein which recommended daily allowance of protein for children. It is also seen to be a rich source of some essential amino acids, fats, vitamins and minerals. The oils and phytochemicals contained in its leaves possess antioxidant and antimicrobial properties amongst others (Itoro, 2015).

Gongronema latifolium is known not only for its high nutritional values but also for its medicinal values due to the composition of different active chemicals in it and also highly useful traditionally as food and its extracts are used in the treatment of malaria, hypertension, diabetes as well as a laxative (Balogun *et. al.,* 2016; Itoro *et. al.,* 2015). It is documented as one of the twenty eight medicinally important leafy vegetables in South Western Nigeria (Ayodele, 2008). A review done by Balogun *et. al.,* (2016) scientifically justified some of its traditional uses in the management of human disease which ranged from hypoglycemic, hypolipidemic, nephroprotective, hepatoprotective, antitumor, broad spectrum antimicrobial, antipyretic, antioxidant,anti-asthmatic, anti-inflammatory, antiulcer, antisickling, mild expectorant, hypolipidemic,analgesic, digestive tonic and laxative properties. Commonly known bacteria such as *Escherichiacoli* (*E.coli*)and *Staphylococcusaureus*(*S.aureus*) are known as intestinal bacteria as they have been involved in several gastro intestinal illnesses. Selvamohan *et. al.,* 2012, reported that some plants like *Ocinum gratissimum* and *Eugenia uniflora* have been reported to be rich in volatile oils and they hold about 75% thymol and possess antimicrobial properties employed in the treatment of ear infection in humans, and also possess antimicrobial effects against *Staphylococcus* sp.,*Shigella* sp. and *Escherichia coli.*

2.LITERATURE REVIEW.

TRADITIONAL MEDICINAL PLANTS

According to World Health Organization (WHO, 2012), traditional medicine also called indigenous medicine is defined as the health practices, approaches, knowledge, and beliefs which uses animals, plants and natural/crude form of medicines, therapies, spiritualism, exercises and techniques, applied in the treatment, diagnosing and preventing illness or to maintain the wellbeing of man. Countries in different continents of the world such as Africa, Latin America, Asia use traditional medicine to attend to some of their primary health care needs (Adaramola-Ajibola *et. al.,* 2017). Okigbo *et. al.,* (2009) reported that traditional medicinal plants are a therapeutic resource used by a large amount of the population in the continent Africa particularly in health care and are acceptable interventions for disease and symptoms (Adaramola-Ajibola *et. al.,* 2017). They could serve also as a starting material for drugs (Okigbo *et. al.,* 2009). Hence, there is a progressive increase in demand for these medicinal drugs both in industrialized nations as well as in developing countries (Abere *et. al.,* 2010).

Various studies have shown the efficacy of edible vegetables against bacteria (Edim *et. al.,* 2012; Eleyinmi, 2007; Enyi-Idoh *et. al.,* 2012). Particularly,*Gongronema latifolium* have been reported to have antimicrobial activity against some species of microorganisms (Enyi-Idoh *et. al.,* 2012) and this antimicrobial activity in the plant shows that it could be useful in both food and non-food systems (Eleyinmi, 2007). The antimicrobial effects of this plant especially on different bacterial infections explain the long history of its use in traditional medicine (Malachy *et. al.,* 2017).

Studies have also shown these scented and medicinal plants serve as storage of therapeutic elements used by many Africans in the treatment of various ailments and diseases like arthritis, high blood pressure, diabetes, malaria, hypertension, mental illness, cancer and human immunodeficiency virus and acquired immunodeficiency syndrome (HIV/AIDS) (Okigbo *et. al.,* 2009; Ugochukwu and Babady, 2002) and their efficiency has also been evaluated. These traditional drugs also known as herbs are potent and they can be used in the stead of Butylated Hydroxytoluene (BHT) and Butylated Hydroxyanisole (BHA) which are synthetic preservatives used in the food industry (Bharti and Vasudeva, 2013).

2.1 CLASSIFICATION OF THE PLANT

COMMON NAME OF PLANT: Amaranth globe
BOTANICAL NAME: *Gongronema latifolium*
KINGDOM: Plantae
PHYLUM: Tracheophyta
DIVISION: Agiosperm
CLASS: Eudicots
SUB CLASS: Asterids
ORDER: Gentinales
FAMILY: Apocinaceae
SUB FAMILY: Asclepiadaceae
GENUS: Gongronema
SPECIE: Latifolium

Gongronema latifolium is a plant that has an extensive variety of wholesome and ethno-therapeutic uses in various tropical African communities (Morebise 2015). It is a shrub that climbs other plants by intertwining with leaves and stems of other plants (Balogun *et. al.,* 2016). *Gongronema latifolium* has a place with the family Apocinaceae (Enyi-Idoh *et. al.,* 2012). It is a palatable wholesome/therapeutic plant mostly found in the rain forest zones in Nigeria and other tropical African nations. The plant has broad spherical leave with a pointed tip like the heart, thick green stalk and light green hairy stems that produces white latex when pluck. It produces yellow flower during its flowering season and turns reddish brown during the late stage. *Gongronema latifolium* can be propagated by stem cuttings or seeds (Edim *et. al.,* 2012). The stem can regenerate when it comes in contact with fertile soil and it flowers in Nigeria between July and August (Edim *et. al.,* 2012)

Figure 2.1*: Utazi leaves*

Source: European journal of medicinal plants (2015)

2.2 USES

G. latifolium is known to have been used for medicinal purposes for the cure of many illness ranging from runny stomach, fever, typhoid, malaria, diabetes, dysentery, nausea, anorexia and many diseases caused by bacteria in Nigeria and other west African nations. The leaves of *G. latifolium* are used as vegetables to prepare soups due to its sweet-bitter flavor, older people eat a lot of it as it is believed to reduce body Fats. The plant is either used fresh, dried or applied as a powdery spice (Enitan *et. al.,* 2017). It is utilized as a spice to support the pancreas (Okafor, 1999).

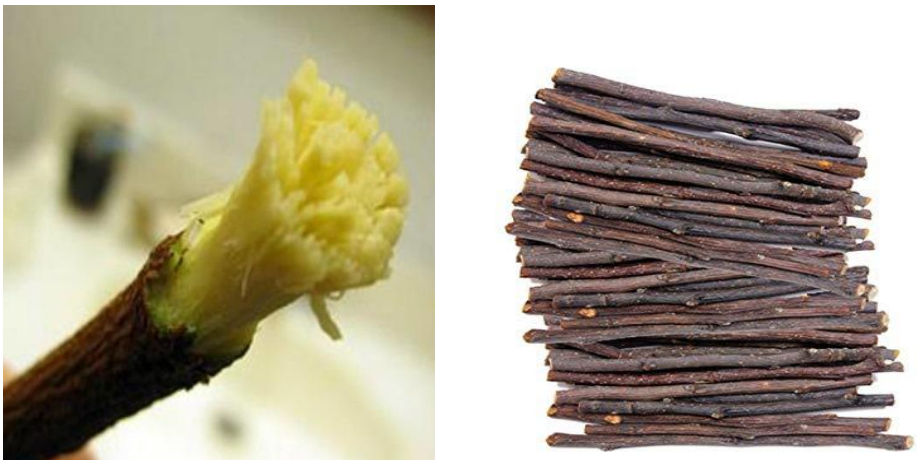

Figure 2.2: Images of Chewing sticks made from G. latifolium

Source: Vanguard Nigeria magazine 2016.

2.3 NUTRITIONAL AND HEALTH IMPORTANCE

*Gongronema latifolium*is highly nutritional vegetable as it is a good source of vitamins, protein, and minerals (Balogun *et. al.,* 2016). The protein content in the leaves show that vegetables is useful in the buildup and body tissue repair process, enzyme and hormone formation and regulation of body processes (Abu *et. al.,* 2014). In the study of mineral and vitamin content of this vegetable, substantial concentrations of retinol and ascorbic acid were found in its leaves (Offor *et. al.,* 2015). *Gongronema latifolium*leaves contain considerable amount of trace elements like zinc, sodium, manganese, iron, phosphorus, high levels of potassium, magnesium and calcium which are important to the health of humans (Offor *et. al.,* 2015). High concentrations of vitamins A, C and E were also found in *Gongronema latifolium* leaves (Atangwo *et. al.,* 2009).

Table 2.1 - Nutritional composition of Gongronema latifolium leaves in percentage

Nutritional composition	Percentage composition
Crude protein	9.8 – 27.2
Lipid extract	6.1
Ash	5.8 – 11.6
Crude fibre	8.7-10.8
Tannin	0.3
Nitrogen free extract	44.3

Source: *African Journal of Biotechnology* 13(50): 4541-4546.

Table 2.2 - Vitamin composition of Gongronema latifoliumleaves in percentage

Vitamin	Composition
Vitamin A (carotene)	360 1µ/100g
Vitamin B_1 (thiamine)	45 1µ/100g
Vitamin B_2 (riboflavin)	290 1µ/100g
Vitamin B_3 (niacine)	0.97%
Vitamin C (ascorbic acid)	0.15%
Vitamin E (tocopherol)	0.82%

Source: *African Journal of Biotechnology* 13(50): 4544-4547.

Table 2.3 - Mineral composition per 100g dry matter

Mineral	Composition (mg/kg)
Potassium (K)	244.8 – 332.1
Sodium (Na)	110 – 113
Calcium (Ca)	115.4 – 154
Phosphorous (P)	125.5 - 326.9
Iron (Fe)	7.8
Zinc (Zn)	13.4
Lead (Pb)	0.2
Cupper (Cu)	2.3 – 43.5
Magnesium (Mg)	53.8
Cadmium (Cd)	0.1
Cobalt (Co)	115.9
Oxalate	70
Ascorbic Acid	187.1

Source: s*African Journal of Biotechnology* 13(50): 4541-4546.

Table 2.4 - Percentage composition of amino acids and fatty acids

Amino acids and fatty acids	Percentage constituents
Stearic acid	4.6
Bohenic acid	3.7
Arachidic acid	2.8
Leucine	8.97
Valine	7.73
Phenylealanin	6.30
Aspartic acid	13.8
Glutamic acid	11.9
Glycine	10.3
Linoleic acid	31.1
Oleic acid	7.1
Linolenic acid	7.1

Source: *African Journal of Biotechnology* 13(50): 4541-4546.

3.MATERIALS AND METHODS

3.1 MATERIALS AND EQUIPMENTS USED

Four different solvents were used and they include; Water, Ethanol, Methanol and Ethyl acetate.*Gongronema latifolium* leaves which is the test material of concern, blender, storage bottles, refrigerator, rotary evaporator (Figure 3.2), anhydrous sodium sulphate while the strains of bacteria used include the gram positive bacteria (*S. aureus*,*B. Subtilis* and *B. cereus*), gram negative bacteria (*E. Coli*) and they were all collected from the school laboratory facility. Antibiotics used are amoxiciline and DMSO (controls). Brain heart infusion Ager (BHI) was used to culture the bacteria. Other materials used include; soxhlet extractor (Figures 3.3& 3.4), GCMS, incubator, autoclave, petri dishes, test tubes, oven, heating mantle (Figure 3.1).

Figure 3.1: A heating mantle

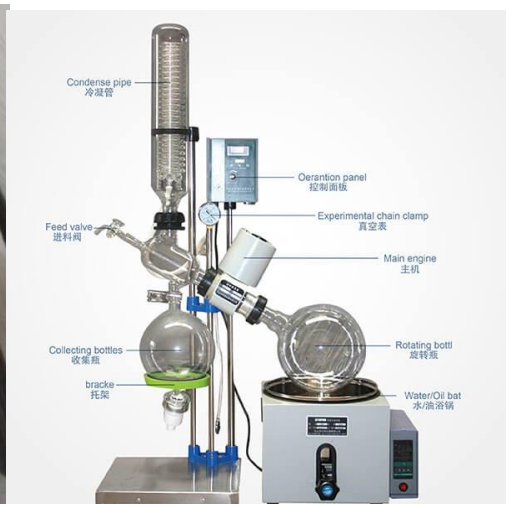

Figure 3.2: A rotary evaporator

Source: wkielab.com

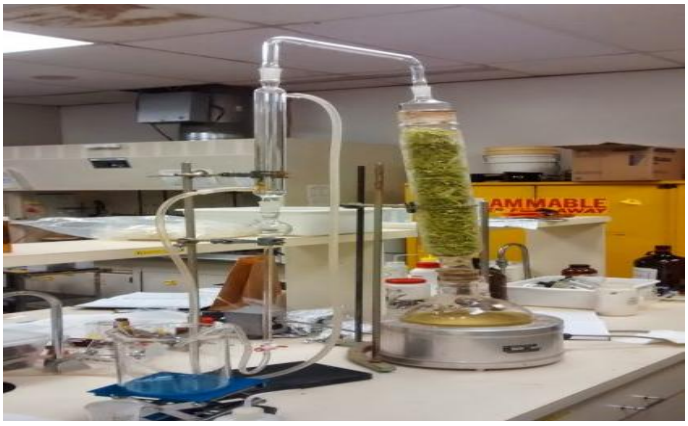

Figure 3.3: Laboratory set up of Soxhlet extractor

Sample preparation

The fresh leaves of *Gongronema latifolium* (sample) was reaped from a local farm in Nigeria, washed under running tap water and air dried at room temperature and under shades until constant weight was achieved. The drying was done under shades to prevent UV light from the sun from damaging the vegetables and reducing its nutrients. It is also dried in this form when storing for future food and consumption purposes.

The sample was ground using a blender and placed in bottles or plastic bags which are stored in a refrigerator until transported to the place of investigation.

Preparation of plant organic extract

100g of *Gongronemalatifolium* leave (sample) was measured and wrapped in a filter paper and the filter paper was then placed in a soxhlet extractor. 500 ml of solvent (water) was added into a round bottom flask and the apparatus.Heat was applied and the solvent evaporated into the soxhlet extractor until the solvent filled the extractor then it drained back into the round bottom flask. This process continued for 6 hours.

Agar preparation (bhi)

In calculating the correct amount of agar needed, according to the manufacturers specification,
52g of agar = 1litre of distilled water

1 petridish = 10ml

Total petri dishes needed x 10ml

6 bacteria to be tested x 5 organic solvent x 10ml = 300ml.

52g = 1 liter (1000ml)

Xg = 300ml

$X = \frac{300\ X\ 52}{1000\ ml} = 15.6g$

15.6g of brain heart infusion agar (BHI) was added into 300ml of distilled water and shaked gently until dissolved. The agar solution was autoclaved at 121^0c for 15 minutes.

Broth reparation (nutrient broth)

Some of the figures used in the calculation below were from the manufacturer's instruction manual.

13g of broth = 1 litre of distilled water

Xg of broth = 150ml of distilled water

$Xg = \frac{150\ X\ 13}{1000\ ml}$

Xg = 3.25g

3.25g of broth was added into 150 ml of distilled water and shaked gently until properly dissolved.

Preparation of 35% dimethyl sulfoxide (dmso)

35ml of liquid Dimethyl Sulfoxide (DMSO) was dissolved in 65ml of distilled water using a conical flask and stored in a refrigerator at -4^0C for further use.

Standardization test of bacteria

The test bacteria was sub-cultured from stock culture on Brain heart infusion (BHI) agar by using a sterile loop to pick colonies of the bacteria growth and inoculating it into a nutrient broth. The sub-culture was incubated for 24 hours in an oven at 35^0C.

Table 3.1 - Standardization test table

Bacteria	Absorbence	Amount of bacteria (in µl)	Amount of sodium chloride (in µl)
E. coli	0.12	50	1900
B. cereus	0.12	600	1200
S. aureus	0.12	800	600
B. subtilis	0.12	500	2000
McFarland solution	0.12	0	0

4.RESULTS AND DISCUSSION ON RESULT

Table 4.1 shows the area of zones of inhibition of the leaf extracts on the disc diffusion method while table 4.2 shows the spectroscopy readings. The methanol extract and the water extract exhibited the highest zones of inhibition on *E. coli*, the methanol extract (MEx) had no effect on *B. cereus* while the ethanol extracts had the highest effects on *S. aureus*. Although, all the extracts had significant effect on *S. Aereus*, the ethanol extract (ETx) had the highest zone of inhibition. The extracts had good effect on *B. subtilis*while ethyle acetate and methanol extracts had the higher zone of inhibition on *B Subtilis*. Comparing with the positive

control used, amoxicillin has the least effects on *B. subtilis* and *B. cereus* with the greatest effects on *E. coli*.

Several studies have reported the antibacterial effects of *Gongronema latifolium*aqueous and methanol leaf extracts on several bacteria isolates especially *S. aureus* and *E. coli* (Eleyinmi, 2007, Oshodi *et. al.,* 2004). Amongst all the extracts tested, the ethanol extract showed a higher antimicrobial effect on the test organisms than the other extracts and this is similar to the study done by Adaramola-Ajibola *et. al.,* (2017) although this conflicts with the study by Amadi *et. al.,* 2018 which showed that methanolic extract had greater activity on the microorganisms tested. The result gotten from this study aligns with the work of Morebise and Fafunso (1998) and Nwinyi *et. al.,* (2008) which showed the antimicrobial activity of the leaf to be higher on *S. aureus* and *E. coli.*

Table 4.1 - Spectrophotometric results

Bactericidal concentration	**EA**	**METH**	**ETH**	**H_2O**	**Bactericidal concentration**	**EA**	**METH**	**ETH**	**H_2O**
EC1	0.036	0.022	0.004	0.008	BS1	0.006	0.023	0.068	0.002
EC2	0.036	0.001	0.005	0.003	BS2	0.008	0.019	0.031	0.0011
EC3	0.062	0.008	0.000	0.000	BS3	0.029	0.048	0.042	0.015
EC4	0.040	0.012	0.012	0.006	BS4	0.028	0.065	0.052	0.022
EC5	0.042	0.041	0.013	0.011	BS5	0.056	0.091	0.075	0.027
C+	0.014	0.009	0.013	0.018	C+	0.010	0.012	0.025	0.011
C-	0.042	0.042	0.036	0.020	C-	0.049	0.096	0.062	0.061
BS1	0.006	0.023	0.068	0.002	BC1	0.058	0.095	0.047	0.034
BS2	0.008	0.019	0.031	0.0011	BC2	0.048	0.101	0.006	0.048
BS3	0.029	0.048	0.042	0.015	BC3	0.060	0.111	0.000	0.039
BS4	0.028	0.065	0.052	0.022	BC4	0.056	0.134	0.055	0.035
BS5	0.056	0.091	0.075	0.027	BC5	0.098	0.156	0.069	0.044
C+	0.010	0.012	0.025	0.011	C+	0.008	0.041	0.028	0.018
C-	0.049	0.096	0.062	0.061	C-	0.218	0.213	0.228	0.194
BC1	0.058	0.095	0.047	0.034	SA1	0.084	0.063	0.099	0.049
BC2	0.048	0.101	0.006	0.048	SA2	0.101	0.081	0.109	0.60
BC3	0.060	0.111	0.000	0.039	SA3	0.102	0.111	0.218	0.97
BC4	0.056	0.134	0.055	0.035	SA4	0.178	0.118	0.128	0.100
BC5	0.098	0.156	0.069	0.044	SA5	0.189	0.104	0.230	0.134
C+	0.008	0.041	0.028	0.018	C+	0.076	0.062	0.169	0.92
C-	0.218	0.213	0.228	0.194	C-	0.285	0.281	0.240	0.266

EA = ethyl acetate extract. METH = methanol extract. ETH = ethanol extract. H_2O = water extract

EC = *E. Coli.* BS = *Bacillus subtilis.* SA = *Staphylococcus aureus.* BC = *Bacillus cereus*

Table 4.2 - Zone of inhibition of the leaf extracts on the isolates

Test isolates	Mex	Wax	Etx	Eax	Amoxicillin
E. coli	8	11	11	11	14
B. cereus	0	11	0.9	1	1.5
S. aureus	7	6.8	10	10	7.6
B. subtilis	0.4	0	0	0	2.1

Zone of inhibiton is measured in millimeters (mm)

Table 4.2 shows the values for the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) against the different bacterial isolates. The minimum inhibitory concentration is an important diagnostic tool as it helps to confirm resistance of microorganisms to antimicrobial agents (Adaramola-Ajibola *et. al.,* 2017). The least MIC was observed in the water and methanol extracts, the lowest MBC was observed in the water (Wax), ethanol, methanol and ethyl acetate (EAx) extract while the highest MIC was observed in the methanol, water and ethane extract, the highest MBC was observed in the hexane, ethanol and methanol extracts.

ABBREVIATIONS

MIC- Minimum Inhibitory Concentration, MBC- Minimum Bactericidal Concentration, MEx – Methane extract
Wax – Water extract, ETx- Ethanol extract, EAx – Ethyl Acetate extract, NDT—Not detectable wıthın the tested range
AMC – Amoxicillin control.

5.CONCLUSION AND RECOMMENDATIONS

Conclusion

The extract from *gongronema latifolium* has showed a spectrum of activity as demonstrated in this study against a selected number of bacteria. This spectrum of activity is due to the presence of bioactive substance and phyto chemicalls that's confirmed to be alkaloids, tanins and saponins. The methanolic extract showed the maximum zone of inhibition to *Escherichia coli* while the ethyl acetate extract showed the least zone of inhibition to *Escherichia coli.* Results gotten from this study has corroborated other studies that have shown significant antimicrobial activities on both gram positive and gram negative microorganisms and this has proven the basis for the plant to be used particularly in the treatment of illness caused by microorganisms.

Recommendation

Further studies should be intensified on improving current knowledge of *Gongronema latifolium* and plans should be made on dissemination of knowledge on proper use and correct dosage of the plant prescribed to people.

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