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Evaluation of the Efficacy of African Basil (*Ocimum gratissimum*) Leaf for Disinfecting Well - water obtained from Ekiti State, Nigeria

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ABSTRACT: The demand for potable and affordable water led to comparative research of the efficacy of *Ocimum gratissimum* Leaf (OGL) extract by collecting well water and treated with OGL extract. Both treated and untreated samples were subjected to physiochemical and bacteriological examinations. Data obtained showed that the pH and feacal coliform counts ranged from 6.17 to 6.74 and 0 to 192 CFU/100 mL for well water sample A; 6.56 to 7.24 and 0 to 118 CFU/100 mL for well water samples B; 6.81 to 7.79 and 0 to 75 CFU/100 mL for well water samples C respectively. The OGL extract includes various bioactive components (i.e. steroids, tannins, etc) according to the phytochemical tests. The first feacal coliform count was above the allowed range (i.e. >50 CFU/100 mL) and the pH value tended to be acidic (6.5), with a colour of >15 H.U. and an unclear (brownish) appearance. The Coliform count was lowered with each dosage of OGL extract until it reached zero count after 24 hours' contact time at an optimum dosage of 5 mL and the total bacteria counts were gone. The beneficial influence (s) from this study confirms the disinfectant potential of OGL extract for well water treatment.

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The use of plants and plant products for water treatments predates the dawn of civilization(Megersa *et al*., 2014). Plants such as *Vernonia amygdalina*, *Moringa oleifera*, *Carioca papaya*, Ginger root and leaf, Water melon etc., have demonstrated effectiveness when subjected to water and wastewater treatment (Bhattacharjee and Gi, 2013; Ghawi, 2017; Evbuomwan *et al*., 2018). Their effectiveness in water treatment is traced to the presence of several bioactive compounds such as steroids, tannins, flavonoids, saponins, terpenoids alkaloids, inulin's, phenolic compounds, Phlobatamins, anthraquinones and cardiac glycosides (Megersa *et al*., 2014; Agholor *et al*., 2018). Groundwater is the principal source of domestic water supply in Ado Ekiti, as the present effort of the State Government in providing potable water to the inhabitance is still a work in progress. Wells either shallow or deep are easily susceptible to various forms of environmental contaminations arising from their proximities to services such as soak-pit, burial sites, refuse dump-sites, pit-latrines, e.t.c. giving rise to the likely occurrences of contaminations with bacteriological contamination been the direst and occurs as water-borne diseases took the form of Cholera, Dracunculiasis, Hepatitis, Typhoid and Filariasis, etc (Panchal and Parvez, 2019; Aribisala *et al*., 2017; Ndububa and Adamolekun, 2017; Agholor *et al*., 2018; Oyedele *et al*., 2019; Oyegoke *et al*., 2020). This research seeks to complement the existing conventional methods of water treatment such as chlorination, boiling, disinfection by ultraviolet (UV), etc. to provide an organic, chemical-free, energy deficient and sustainable option, through the use of *Ocimum gratissimum* leaf aqueous extract as a form of disinfectant to sterilize water (Juran and MacDonald, 2014; Fagerli *et al*., 2017).*Ocimum gratissimum* as a plant is used widely in the traditional medicinal practices in many countries. It is used in the treatment

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of epilepsy, high infection, diarrhoea, management of the baby's cord, to keep the wound surfaces sterile, also used in the handling of fungal infections, fever, cold and catarrh. Hence, the objective of this work is to evaluate the potentials of the efficacy of African Basil (*Ocimum gratissimum*) Leaf in disinfecting well-water obtained from Ekiti State, Nigeria.

MATERIALS AND METHODS

Collection and processing of Ocimum Gratissum (OG) plant: Ocimum gratissum plant is a member of the *Lamiaceae* family locally known with different names in Nigeria: Ebira (Ireru), Hausa (Dai doya ta Gida), Ibo (Nchuanwu / Ahuji), Nupe (Tan - motsungi-wawagi), and Yoruba (Efinrin) (Agholor *et al*., 2018) were picked from a local farm at Omisanjana Ado-Ekiti in Ekiti Central Senatorial District, Ekiti State, the Southern part of Nigeria as shown in Figure 1. The plant leaves were identified in the Agricultural Technology laboratory in Federal Polytechnic Ado-Ekiti. The identified OG was subjected to cleaning, washing, stripping leaves from stems and dried at room temperature for 5 days as shown in Figure 3. After complete drying of the plant leaves, it was pounded (using mortar and pestle) and later blended (using electric blender machine) into fine powdered for phytochemical screening.

Fig 1: Picking of Ocimum gratissimum Plant

Fig 2: Picked, washed and dried Plant Leaves

Fig 3: Fluid extracted from OGL

Ocimum gratissimum fluids were extracted by squeezing the plant in a clean plastic container for about 30 - 45minutes, the fluid or extract obtained was filtered out through a 200 micron sieve in other to remove residue as depicted in Figure 3.

Collection of well water sample: Samples from well water were randomly obtained from wells within Ado-Ekiti Metropolis. These samples were subjected to a bacteriological test to ascertain their bacteriological positions. Samples found to have feacal coliforms above the WHO (2012) acceptable limit of 0 CFU/100ml were used for this research.

Phytochemical studies of Ocimum Gratissimum: The method adopted is in line with Panchal and Parvez (2019), Nn (2015). Phytochemical means "plant chemicals". These are the inherent chemical properties of the plant (Singh, 2006). The aqueous extract of *Ocimum gratissimum* was subjected to phytochemical analysis to access the presence or absence of phytochemical constituents.

Test for the presence of Alkaloids (Wagner's test): 10 gram of the *Ocimum gratissimum* powder was measured into a beaker and 100 mL of distilling water was added and left for 30 minutes, afterwards filtered to remove the residue. Wagner's reagent was prepared by dissolving 2 gram of iodine and 6 gram of KI (potassium iodide) in 100 mL of distilling water. A few drops of Wagner's reagent were added to about 2 mL of OGL extract along the sides of a test tube. A reddish-brown precipitate indicates the presence of alkaloids and confirms the test as positive.

Test for the presence of Anthraquinones (Borntrager's test): 0.5 to 1 gram of the extracts were boiled with 10% HCl for a few minutes in a water bath, filtered and allowed to cool. An equal volume of chloroform was added to the filtrate. Two drops of ammonium sodium were added to the mixture and heated. The

formation of red-rose colouration confirms the test as positive showing the presence of anthraquinones.

Test for the presence of flavonoids: 2 mL of the fluid extract in alkaloids above was added into a test tube and 2 mL of sodium hydroxides was added and heated for 3 - 4 minutes afterwards dazed with 1 mL of dilute ammonia solution, yellow colouration of the solution was detected and confirms the test as positive for the presence of flavonoids.

Test for the presence of Phlobatamins: OGL aqueous extract was boiled with 1% aqueous hydrochloric acid (HCl) to confirm the deposition of the red precipitate and confirms the test as positive.

Test for the presence of Glycosides (phenolphthalein indicator's test): 4 gram of sodium hydroxide (NaOH) was weighed and diluted in 100 mL of distilling water. OGL plant powder of 10 gram was weighed in a beaker and dissolved in 100 mL of distilling water and allowed to wait for 30 minutes to settle before been filtered. 5 mL of this solution was taken and 2 drops of phenolphthalein indicator were added. 10 mL of sodium hydroxide solution was added to the mixture to observe a brick-red precipitate as an indication for the presence of glycosides.

Test for the presence of Saponins (Frothing test): 0.2 gram of OGL extract was added into a test tube and shacked with 2 mL of distilled heated to boil. The frothing appearance of a layer of small bubbles (creamy) showed the presence of saponins.

Test for the presence of steroids (Salkowski test): Two drops of concentrated sulphuric acid were added to 1 mL of OGL aqueous extract. The presence of red colouration precipitation occurs and indicates the presence of steroids.

Test for the presence of Tannins (Ferric chloride test): The presence of tannins was tested in 0.5 gram of the OGL powder and was stirred with 10 mL of distilled water. The extract was filtered; ferric chloride reagent, alcoholic acids and 10m of distilling water were added to the filtrate. A blue-black precipitate was observed as evidence for the presence of tannin in the sample.

Test for the presence of Terpenoids (Salkowski test): The presence of terpenoids was tested in 0.2 g of the OGL aqueous extract, mixed with 2 mL of chloroform and concentrated sulphuric acid (3 mL H_2SO_4) to form a layer. A reddish-brown colouration of the interface was formed to indicate positive results for the presence of terpenoids.

Test for the presence of phenol: The presence of phenol was tested in 2.5 mL of the OGL extracts in a test tube. 2 drops of ferric chlorides were added into the test tube (extracts) and shacked well; reddish-brown precipitation occurs which indicated the presence of phenol.

Application of Ocimum gratissum Leaf (OGL) extract to the contaminated well water samples: 100 mL of water sample from each well was measured in 6 (six) different glass beakers, labelled as number 0 to 5. These were arranged in three sets with number 0 as a control. Varying OGL aqueous extract of 1, 2, 3, 4 and 5 mL were pipetted into the beakers containing well water samples. The mixture was stirred thoroughly using an Electromagnetic Stirrer Machine set to volume number 5 and given a 60 minutes contact time.

Feacal Coliform Culture (Pour Plate Method): Feacal Coliform count was determined by weighing 8 grams of EMB Agar and dissolved in 220 mL of distilled water in a conical flask of 250 mL capacity and place on Magnetic Stirrer machine to stir for about 10 minutes for well mixed. The mouth was covered with aluminium foil and cotton wool, and then it was heated up to 121 ^{o}C in an autoclave. 1 mL of the solution (OGL extract and well water sample) was poured into a petri dish. The molten EMB Agar was poured into the solution in the petri dish and allowed to solidify or cool to about 45 ^{o}C. The incubator was sterilized and set at 37 ^{0}C. The petri dish with samples was carefully arranged inside the incubator and incubated for 48 hours. The bacteriological analysis or microbial counts (colony counts) which form or grew on the plates were counted with colony counter (Funke Gerber Colony counter apparatus) and the values were recorded as per plates or Petri dishes.

pH Measurement: The pH reading of all samples was carefully taken using the Hanna Digital pH meter(SON, 2007). The pH meter was calibrated with buffer solution of pH of 7. The pH values of the samples were determined by immersing the pH meter into the sample and taking a reading, removed, rinse with distilled water and inserted in the buffer solution before running the test on other samples and the value was recorded. The procedure was repeated for all samples taken (Note: 0 - 6.9 = Acidic water; 7 = Neutral and 7.1- 14.0 = Alkaline water).

Colour Measurement: The colour values of the samples were measured using the Hazen Unit (HU) colour comparator apparatus (SON, 2007).

The right-hand side test tubes in the apparatus were filled with water samples while the second tube was

with de-ionized water. The tubes were inserted into the colour comparator apparatus.

The graduated Hazen colour disc attached was rotated until colour matches. The reading corresponding to the colour match is taken as the water colour value. The acceptable limit for potability is between 5 and 15 HU. The higher the value the more polluted is the sample.

Appearance Measurement: The appearance of the samples was determined by measuring the sample half-filled in a test tube and visually noting its characteristics, e.g. clear, sparkling, cloudy, foaming, etc.

RESULTS AND DISCUSSION

Phytochemical analysis of Ocimum Gratissum Leaf (OGL): Table 1 presents results of the phytochemical analyses on the OGL.

It showed that there was presence of all the secondary metabolites (i.e. phyto chemicals) tested, which are steroids, tannins, flavonoids, saponins, terpenoids, alkaloids, and phlobatamins.

Thus, there is possibility OGL eliminating many unwanted chemicals, metals and microorganisms due to presence of these functional groups (phyto chemicals) in it (Adetoro and Ojoawo, 2020).

Physicochemical and bacteriological tests: pH assessment*:* From Table 2, the pH concentrations of sample A reduced from the initial value of 6.74 for control to 6.48, 6.45, 6.38, 6.22 and 6.17 respectively as the OGL extract increases in dosages from 0 to 5 mL per 100 mL of water solution.

This trend can also be seen in samples B and C. Increase in the dosages of OGL extract negatively impacted the pH concentrations of the samples, which tends toward the acidic state as against the WHO (2012) recommendation.

Colour measurement: The introduction of increasing dosages of OGL extract into the well-water A, B and C negatively affected them as seen in Table 2.

The colour values increased from the initial values of 10 HU in sample A, 15 HU in sample B and 15 HU in sample C to a value of 50 H.U as against the recommended WHO (2012) value of 5 – 15 HU.

Table 1: Phytochemical constituents of Ocimum gratissimum Leaves (OGL) extract

S/No	Parameters	Test	Remark
1	Alkaloids	Meyer's test	++ve
2	Steroids	Salkowski test	++ve
3	Anthraquinones	Carbon tetrachloride tests	+ve
4	Saponins	Frothing tests	++ve
5	Phenol	Ferric chloride tests	+ve
6	Flavonoids	Ferric chloride tests	+ve
7	Tannin	Ferric chloride tests	++ve
8	Glycosides	Keller's tests	++ve
9	Terpenoids	Salkowski test	+ve
10	Phlobatamins	Ferric chloride tests	+ve

Note: ++ve… more presents (positive). +ve… slightly presents (positive). –ve… slightly absents (negative). --ve…absents (negative)

Appearance: The appearances of all the samples (A, B and C) were negatively impacted as the appearances were reduced from clear in sample A, cloudy in sample B and clear in sample C to brownish as the dosages increased from 1 to 5 mL in all the samples as described in Table 2.

Table 2: Summary of all the selected tests carried out on the mixture of OGL extract and well water samples

OGL (mL)	Well Water Sample A				Well Water Sample B				Well Water Sample C			
	pH	Colour (HU)	App.	Colony counts (CFU / 100mL)	pH	Colour (HU)	App	Colony counts (CFU / 100mL)	pH	Colour (HU)	App	Colony counts (CFU / 100mL)
0(control)	6.74	10	Clear	192	7.24	15	Cloudy	118	7.79	15	Clear	75
1	6.48	50	Brownish	100	6.97	50	Brownish	29	7.41	50	Brownish	13
2	6.45	50	Brownish	11	6.77	50	Brownish	11	6.98	50	Brownish	3
3	6.38	50	Brownish	1	6.73	50	Brownish	1	6.92	50	Brownish	0
4	6.22	50	Brownish	0	6.69	50	Brownish	0	6.87	50	Brownish	0
5	6.17	50	Brownish	0	6.56	50	Brownish	0	6.81	50	Brownish	0
WHO (2012) Limits	>6.5 < 8.5	> 5 < 15	Clear	0	>6.5 < 8.5	> 5 < 15	Clear	0	>6.5 < 8.5	> 5 < 15	Clear	0

Bacteriological investigation: Aqueous extract of OGL positively impacted the bacteriological composition of all the samples. The coliform count was reduced from the initial count of 192 / 100 mL in sample A, 118 CFU / 100 mL in sample B and 118 CFU / 100 mL in sample C, to 0 CFU / 100 mL count in all the three samples as the dosages increased to a maximum of 5 mL / 100 mL. Panchal and Parvez

(2019), Evbuomwan *et al.*, (2018) opined that the phytochemical presence of secondary metabolites such as alkaloids, steroids, anthraquinones, saponins, phenol, flavonoids, tannin, glycosides terpenoids and phlobatamins, might be responsible for the observed antibacterial activity of the OGL extract.

Conclusion: Based on the above results and discussions, the OGL extract was unable to address the pH values, colour and appearances of the well water samples in line with the stipulated requirements; but it was useful in the removal of feacal coliform (especially *E. coli*) present in all the water samples. This revealed the antibacterial activity or effects of OGL extract. Therefore, it could be concluded that OGL extract is suitable for the sterilization of water and may be used alongside with other conventional methods of water treatment.

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