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PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF THE WET AND DRY FLOWERS OF NIGERIAN GROWN CLOVE BASIL (*Ocimum gratissimum*)

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ABSTRACT

The dry and wet flowers of Ocimum gratissimum were collected within the premises of Federal Government Technical College, Saki. The non volatile extracts were obtained by maceration cold method using water as extractant. The phytochemical analysis was done using classical method of analysis. Antimicrobial activity (sensitivity test) was carried out using the disc method on a dilute streaked culture media. Phytochemical screening of the non volatile extract obtained from dry flower showed the presence of alkaloid, cardiac glycosides, phlobathatin, terpenoid, flavanoid and saponin, while that of wet sample displayed the presence of alkaloid, cardiac glycoside, tannin, terpenoid and saponin. The non volatile extract of dry

Introduction:

Ocimum gratissimum belongs to the family *lamiaceae* . It is also known as clove basil. It is a flowering plant also known as mint (Raymond *et al.*,2004). *Lamiaceae* family are known for their sedative, diuretic, tonic, antispamodic and antiseptic properties (Ramasubramania and Raja, 2012).

O.gratissimum belongs to the group of plant known as spices (Vierra and Simon, 2000). It has local name of Efinrin in Yoruba, Diadoyal in Hausa and Nchuanwu in Igbo (Owulade, 2004). It acts as insecticide, nematicide, fungicide and antimicrobial (Effraim *et al.,* 2003). The biological activities of this

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flower sample inhibited Staphylococcus aureus, Escherichia coli and Klebsiella pneumoneae. It was observed that non volatile extract of the wet flower sample inhibited Klebsiella pneumoneae, Escherichia coli but not Staphylococcus aureus, hence, the dry flower sample displayed better antibacterial activity than the wet flower extract. It is concluded that the aqeous extract of the plant flower is a potential source of antimicrobial that could be used in treatment of diseases caused by Klebsiella pneumoneae, Escherichia coli and Staphylococcus aureus.

Keywords: *antimicrobial, phytochemicals, staphylococcus, terpenoids,*

Plant are attributable to the phytochemicals present in it. They help plant to thrive or thwart competitors, predators or pathogens.

Phytochemists study phytochemicals by first extracting and isolating compounds from the origin plants, define their structures or test in laboratory model systems such as cell cultures, vitro experiments or vivo studies using laboratory animals (Molyneux *et al.,*2007). They may be extracted using different methods such as maceration, infusion and decoction, soxhlet extraction or hot continous extraction (Altemimi et al., 2017), supercritical fluid extraction (Capuzzo *etal.*,2013), microwave assisted hydrodistillation (Farhat *et al.*, 2009), ultrasound assisted extraction (Filly *et al.,* 2014).

Varieties of methods have been used for the structural elucidation of phytochemicals such as instrumentation methods and classical laboratory methods.

Classical laboratory techniques uses various reagents to carry out test such as liebermann burchard's reagent, molish reagent, Drangendoff's reagent, Mayer's reagent and some other reagents. Ability of the sample to react with these reagents to give the expected observations shows the presence of these phytochemicals such as tannin, saponin, flavanoid, cardiac glycosides, steroids, alkaloids, terpenoids, phlobathanin etc (Alexandra, 2016). Presence of these phytochemicals makes the sample antibacteria, antiviral, antitumor etc.

MATERIALS AND METHOD

The dry and the wet flowers of *O.gratissimum* was collected from its stalk by handpicking at Government Technical College, Saki. The sample was weighed (30g), crushed using mortar and pestle, soaked in a beaker with 100ml of water and left to stand for 24hours (maceration cold method) using water as extractant .

The nutrient agar was prepared by weighing of 28g of the agar powder and dissolving it on 1000ml of Distilled Deionized Water, stirred vigorously for thorough mixture. The resultant solution was then autoclaved at 15 pound pressure at 121oC for 15 minutes. It is then poured hot into petri dishes and carefully left to solidify.

After solidification, inoculation was carried out in a disinfected innoculating room. Pure isolates were picked with sterilized loop and cultured on an agar plate using dilute streaked techniques. Culturing was done near a spirit lamp to avoid contamination. The bacteria ie *Klebsiella pneumoneae, Staphylococcus aureus and Escherichia coli.* The stock culture of these bacteria were procured from Microbiology Laboratory, The Oke- Ogun Polytechnic, Saki. Viability and purity of the culture were checked before maintaining them on a nutrient agar slopes.

ANTI- BACTERIAL ACTIVITY

Whattman no.1 filter paper was perforated to get a disc shape which was then wetted with one drop of the plant extract which is equivalent to 30mg/ml and it was air dried near the spirit lamp. It was placed on the streaked plate. The resulting media was incubated for 24hours at 37^0C. Analysis was done in duplicate.

DETERMINATION OF PHYTOCHEMICAL CONSTITUENTS

TEST FOR TANNIN

2ml of the extract was measured using measuring cylinder into a test tube. A few drops of 0.1% ferric chloride was added for observation of brownish green or a blue black coloration (Alexander, 2016).

TEST FOR SAPONIN

5ml of the plant extract was diluted with distilled water up to 20ml and this was shaken for 15minutes in a graduated measuring cylinder. The formation of 2cm thick foam indicated the presence of saponin (Banu and Catherine, 2015).

TEST FOR FLAVANOIDS

2ml of 10% dilute Ammonia solution was added to a portion of the aqueous filterate of the plant extract, followed by addition of concentrated H_2SO_4. The observation of a yellow coloration in the extract indicated the presence of flavanoids (Alexander, 2016).

LIEBERMANN- BURCHARD'S TEST

A small amount of the extract (1ml) was dissolved in 2ml of acetic anhydride and few drops of concentrated sulphuric acid was added. An array of color change shows the presence of phytosterols (Raaman, 2006).

MOLISH'S TEST

Two drops of alcoholic solution of analar Naphtol was added to 2ml of filterate and 1ml of concentrated sulphuric acid was added slowly along the side of the test tube. A violet ring indicated the presence of carbohydrates (Gusthinnadura, 2017).

MAYER'S TEST

Two drops of Mayer's reagent was added along side of the test tube into few amount of plant extract. The presence of alkaloids was indicated by a white creamy precipitate (Banu and Catherine, 2015) .

DRAGENDROFF'S TEST

The addition of few drops of Dragendroff's reagent into the extract gave red precipitate if alkaloids are present in the sample (Gusthinnadura, 2017).

TEST FOR CARDIAC GLYCOSIDES

2ml of the extract was treated with 2ml of glacial acetic acid containing 1 drop of Ferric Chloride solution (0.1%) was underlayed with 1ml concentrated H_2SO_4. A brown ring of the interface was indicated by a deoxy sugar characteristic of cardenolides. The violet ring did not appear below the ring, while in the acetic layer, a greenish ring was not formed throughout thin layer (Alexander, 2016).

TEST FOR PHLOBATANIN

Aqueous extract of the plant sample (2ml) was boiled with 1% aqueous hydrochloric acid and deposition of a red precipitate was seen as an evidence for the presence of phlobatanins (Alexandra, 2016).

TEST FOR TERPENOIDS

2ml of the extract was mixed with 2ml of chloroform and 3ml of concentrated Hydrochloric acid was added to form a layer. A reddish brown coloration of the interface was formed to show the positive result for the presence of terpenoids (Alexandra, 2016).

TEST FOR ANTHRAQUINONE

2ml of the extract was boiled with 10ml of H_2SO_4. The mixture was then shaken with 5ml dilute ammonia and colour change was observed.

RESULTS

The result of phytochemical constituents of the dry and wet flowers of *Ocimum gratissimum* are as shown in Table 1 while the result of antibacteria activity are as shown in Table 2.

Table 1 : The phytochemical constituents present in wet and dry flower extracts of *Ocimum gratissimum*

Test.	Wet flower.	Dry flower
Alkaloid (Drangendroff)	-	-
Alkaloid (Mayer)	+	+
Resins	-	-

Cardiac glycosides	+	+
Tannins	+	-
Steroids	-	+
Phlobatanin	-	+
Anthraquinone	-	-
Terpenoids	+	-
Flavanoids	-	+
Saponin	+	+

Key: - means absent, + means present.

Table 2 : Anti-microbial activity of the flower extracts.

Organisms.	Wet flower (mean in mm)	Dry flower (mean in mm)
Klebsiella pneumoneae.	26.67.	29.21
Staphylococcus aureus.	0.00.	24.21
Escherichia coli.	16.51.	25.40

DISCUSSION

The phytochemical analysus results (Table 1) *Ocimum gratissimum* wet flowerllp contained alkaloids, cardiac glycosides, tannin, terpenoids and saponin which conformed with research work carried out by Sandeep, 2017 in aqueous extract and Akinmoladun *et al.,* 2007 in methanolic extract of *Ocimum gratissimum* leaf . Presence of these compounds indicates that the wet flowers had antimicrobial potential because of the presence of the alkaloid, tannin and terpenoid.

Ocimum gratissimum dry flower extract revealed presence of alkaloid, cardiac glycosides, phlobatannin, flavanoid and saponin which conform with research work done on the plant extract carried out by Chetia *et al.,* 2014 and Alexander, 2016. Dry flower also showed antimicrobial effects because of the presence action of alkaloid and flavanoid (Prashant *et al.,* 2011).

Both can be used to treat diarrhoeal and anthelminthics due to the presence of alkaloid and saponin in both, tannin in wet and flavanoid in dry. The extracts have tendency of preventing cancer due to the presence

of saponin as reported by Preshant *et al.,* 2011, but better performance would be expected from dry sample extract due to synergistic action of flavanoid and saponin.

The antibacterial screening of dry and wet flower of *Ocimum gratissimum* (Table 2) shows that both had an inhibitory effect on *klebsiella pneumoneae* and *Escherichia coli*. Only the dry sample displayed inhibitory effect on *staphylococcus aureus*. Hence, the flower extract can be used for treatment of diseases caused by the screened bacteria. Therefore, aqueous extract of the dry flower can have higher growth inhibition against the tested bacteria than the wet flower. These result conform with Abdullahi, 2012 and Olanrewaju and Uju, 2014.

The t-test value shows that at 95% probability level, there is no significance difference in the inhibitory zones of wet and dry flower extracts against the tested bacteria. Hence they could be used interconvertibly although dry is more active than wet flower.

CONCLUSION

This study shows that *Ocimum gratissimum's* flower extract can be used as an antibacterial when there no enough leaves. The flowers (dry and wet) have phenolic compounds such as saponins, tannin etc. which are responsible for the high anti oxidant activity of the flower extracts hence; they can easily inhibit micro- organisms. However, dry flower is more active than wet flowers because of the presence of flavanoids in dry flower extract hence; dry flower extract has higher inhibitory ability than wet flower extract. It is hereby recommended the use of wet and dry flower extracts of Oc*imum gratissimum'a*s an alternative antibacterial agents against *Klebsiella pneumoneae, Staphylococcus aureus* and *Escherichia coli.*

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HARNESSING THE POTENTIALS OF MICRO-ORGANISMS AND PLANTS AS CLEANUP AGENTS FOR PROTECTION AND RESTORATION OF A SUSTAINABLE SOCIETY

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ABSTRACT

The environment has suffered many severe treatments as a result of anthropogenic activities resulting to contaminated environment. Environmental Biotechnology has the potentials of boosting healthy living, societal and economic development of a nation. This study focuses on how some micro-organisms and plants are engineered to cleanup contaminated environment to enhance sustainability of the occupants. Environmental remediation using microbiological and plant intervention in waste water treatment, solid waste treatment; soil treatment and waste gas treatment are examined. It is therefore, recommended that pollution should be prevented in order to provide unprecedented benefits and as well adopts production method that are more economic in energy and resource

Introduction:

Biotechnology is the integration of natural sciences and engineering in order to achieve the application of organisms, cells, parts thereof and molecular analogues for products and services (Van Beuzekom and Arundel, 2006). Biotechnology is versatile and has been assessed a key area which has greatly impacted various technologies based on the application of biological processes in manufacturing, food processing, medicine, agriculture, environmental protection and resources conservation. The new wave of technological changes has enhanced dramatic improvements in various sectors such as production of drugs, steroids, vitamins,

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