

Anti-Inflammatory and Antimicrobial Activities of Powered and Ethanaol Extracts of Scent Leaf (*Ocimum gratissimum*) and Curry Leaf (*Murraya koenigii*)

Onyeyilim Ebuka Leonard¹, Ezeokonkwo Mercy Amarachi¹,
Uzoewulu Chiamaka Peace¹, Eze Florence Uchenna¹,
Urama Festus Chinonso¹, Eze Cosmas Chinweike¹, Okonkwo Vivian Ifeoma²
Otunomo Idowu Lyabo¹

¹Department of pure and Industrial Chemistry University of Nigeria, Nsukka

²Department of Science Laboratory Technology University of Nigeria, Nsukka

ebuka.onyeyilim@unn.edu.ng

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Abstract. In this manuscript, the authors explore the antimicrobial and anti-inflammatory properties of extracts from two commonly found and used leaves from species *Murraya koenigii* and *Ocimum gratissimum* L., Ethanolic extracts were obtained by maceration and were then concentrated. Serial dilutions of the extracts were done for testing. The extracts were tested for growth inhibition of four bacterial and two fungal strains. Visual examination of growth inhibition, compared to standard antibiotic controls, provided minimum inhibitory concentrations for the two extracts against the six microbial strains. In another experiment, the authors test the anti-inflammatory properties of the two leaf extracts in the standard rat-paw model of inflammation. Results from growth inhibition studies suggest that the two leaf extracts inhibit the growth of all six microbes, with varying levels of sensitivity. Results from anti-inflammatory studies are more impressive, with leaf extracts inhibiting inflammation at levels competitive with the standard drug, indomethacin.

Introduction

Infectious diseases are nowadays the second major cause of death worldwide and the third leading cause of death in developed countries [1]. Also, microorganism resistance to multiple antimicrobial agents has become a serious problem [2,3]. Scent leaf (*Ocimumgratissimum*) is a well-known medicinal plant which has received a great deal of attention over the past few decades around the world [4].

Curry leaves (*Murrayakoenigii*) also are a popular leaf-spice used in very small quantities for their distinct aroma due to the presence of volatile oil and their ability to improve digestion. These leaves are widely used in Asian and African cuisines for flavouring foods. The leaves have a slightly pungent, bitter and feebly acidic taste, and they retain their flavour and other qualities even after drying [5]. The Seeds and leaves yield an essential oil containing eugenol, it is used widely in perfumes, cosmetics, pharmaceuticals and confectionary industries [6]. Extracts of *Ocimum gratissimum* contain many phytochemicals which are widely used for medicinal purposes. The administration of aqueous extract of both the curry and olive leaves has shown a significant decrease in the blood glucose level in STZ-induced diabetic rats [7]. Extracts from *Ocimum gratissimum*. Plants have shown strong inhibitory effects on HIV-1reverse transcriptase and platelets aggregation [8,9].

Most works have been done on these leaves but no work concerning the antimicrobial and anti-inflammatory activities of these leaves have been carried out. This work therefore, is intended to extract the chemical constituent of the leaves of *Ocimumgratissimum* and *Murrayakoenigii*, using ethanol as a solvent for a portion and obtaining the solid extract of another portion with the view to authenticating the antimicrobial potentials and anti-inflammatory activity of these extracts against some harmful pathogenic bacteria and inflammation.

Materials and Methods

Preparation of Ethanol Extracts for Antimicrobial Test

The antimicrobial test was carried out by the method adopted by [10 and 11]. The fresh leaves of *Murrayakoenigii* and *Ocimum gratissimum* were washed with tap water, dried in the lab under ambient temperature and powdered by a mechanical grinder. The dried powders were then extracted with ethanol to give their extracts respectively. The plant leaves about 10g each was sequentially extracted with ethanol (150ml) by maceration process for 5 days at room temperature. The obtained extracts were filtered by using whatmann filter paper. The extracts were concentrated using a rotary evaporator at 60°C evaporated to obtain syrupy solution weighing 19.8g and 19.2g for curry leaves and Scent leaves respectively. This was put in an air-tight container and stored in a refrigerator for further analysis.

Preparation of Solid Extracts for Anti-Inflammatory Test

This test was carried out using the method reported by [12]. The fresh leaves of *Murrayakoenigii* and *Ocimumgratissimum* L. were washed with tap water, dried in the lab under ambient temperature and powdered by a mechanical grinder. The powdered plant materials were filtered to obtain the extract, which was stored in an air-tight container and stored for further analysis.

Evaluation of Antimicrobial Activity

The antimicrobial sensitivity testing of both crude and prepared concentrations of the plant extracts was determined using the agar-well dilution method.

Test Micro-Organisms

Six standard clinical isolates used in this work were obtained from laboratory stock organisms in the Department of Pharmaceutical Microbiology, University of Nigeria, Nsukka. They include four bacteria, -*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhi* and two Fungi, - *Candida albicans*, and *Aspergillusniger*. These micro-organism are selected because they have been implicated to be among the organisms causing enteric diseases/infections and easily develop resistance to commonly prescribed antibiotics.

Preparation and Sterilization of Materials

All glass wares used were first soaked in detergent solution for about 30 minutes, then washed and rinsed with clean water and allowed to dry. The test tubes were plugged with cotton wool, while the pipettes and petri dishes were packed in a canister. All these were sterilized using hot air oven at 160°C for one hour.

Preparation of Culture Media

Nutrient agar: A 28g of nutrient agar powder was dissolved in 1000ml of distilled water, and was allowed soaking for 10mins. The agar suspension was brought to melt by boiling in a water bath. A 20 ml aliquot of the molten nutrient agar was dispensed into a bijou bottles, cocked, and sterilized in an autoclave at 121°C for 15mins. The sterile molten nutrient agar was stored at 42°C until use.

Potato Dextrose Agar (PDA): A 47g of PDA powder was dissolved in 1000ml of distilled water, and was allowed soaking for 10mins. The agar suspension was brought to melt by boiling in a water bath. A 20ml aliquot of the molten PDA was dispensed into a bijou bottles, cocked, and sterilized in an autoclave at 121°C for 15mins. The sterile molten potato dextrose agar was stored at 42°C until use.

Preparation of Test Micro-Organism

Four standard laboratory isolates; *S. aureus*, *E. coli*, *B. subtilis* and *S. typhi* and two fungi; *A. niger* and *C. albicans* (standard laboratory isolates), were all obtained from Pharmaceutical Microbiology Laboratory, UNN.

The pure samples of each test micro-organism were transferred into a sterile petri dish with the aid of sterile inoculating loop, and incubated at 37°C for 24 hours for bacteria and at 25°C for 48 hours for fungi.

Standardization of the Test Organism Suspension:

The micro-organisms were standardized using 0.5 MacFaland turbid equivalent.

Preparation of Sterile Water:

A 200ml of distilled water was dispensed in conical flask and the flask was plugged with cotton and foil. The water was sterilized in an autoclave at 121°C for 15 minutes.

Preparation of the Different Concentration of the Extract Used:

A 5mg/ml (5000µg/ml) stock concentration of the crude extract was prepared by dissolving 10mg of the extract in 2ml of 50% DMSO. 1.0mg/ml, 0.9mg/ml, 0.8mg/ml, 0.7mg/ml, 0.6mg/ml, 0.5mg/ml, 0.4mg/ml, 0.3mg/ml, 0.2mg/ml, 0.1mg/ml, concentrations were obtained using $C_1V_1=C_2V_2$ formula.

Control test (standard):

The standard antibiotic used is Ciprofloxacin, while Fluconazole is the standard antifungi drug.

Experimental

A 4.0ml of crude extract suspension of stock concentration 50mg/ml was transferred to the sterile Petri dish, a 16.0ml volume of double strength sterile molten agar was transferred to the same plate to mix uniformly thus, 1mg/ml concentration was obtained. The other concentrations 0.9mg/ml, 0.8mg/ml, 0.7mg/ml, 0.6mg/ml, 0.5mg/ml, 0.4mg/ml, 0.3mg/ml, 0.2mg/ml, 0.1mg/ml, were obtained using the same $C_1V_1=C_2V_2$ formula. The molten agar plates with different concentrations of the extract were allowed to gel. The plates were divided into seven equal parts with permanent marker. The test microorganisms were streaked on the segments, and labelled. The culture plates were incubated in inverted position at 37°C for 24hours, and at 25°C for 48hours. After the due period of incubation, the plates were observed for sensitivity and resistivity of the organisms to the agents, and the observation was recorded. The plates were further incubated for another 24hour at 37°C, and 48hours at 25°C to determine whether the activity was bacteriostatic or bacteriocidal. The observation was also recorded.

Evaluation of Anti-inflammatory Activity

Albino rats were divided into four groups of six animals each. Oedema was produced by injecting 0.2 ml of a solution of 1% carrageenan in the hind paw. The rats were injected intraperitoneally with 1ml suspension. Group I served as toxicant control, received carrageenan (0.1 ml of 1% solution), group II served as standard, received indomethacin (10 mg/kg, p.o), group III and IV were treated with 100 mg / kg & 100 mg/kg of solid extract of scent leaf and curry leaf respectively by oral route. Note that after the carrageenan was injected into the rats, they were left for about 48 hours to allow the carrageenan to circulate proportionally in the rats causing the inflammation to spread rapidly.

One hour after drug or test compound (extract) administration, 0.1 ml. of 1% carrageenan in distilled water was injected into the sub plantar region of right hind paws of all groups. The paw oedema volume was measured with the help of plethysmometer at zero hour (immediately after injecting carrageenan). The same procedure was repeated at 1, 2, 3 and 4 hour after carrageenan injection. The difference between the initial and subsequent reading gave the actual oedema volume. Reduction in paw volume compared to the control animals was considered as anti-inflammatory response [12]

Percentage Inhibition = $[V_c - V_t/V_c] \times 100$

Where, V_t = mean paw volume of test group & V_c = mean paw volume of control group.

Control Test (Standard):

The standard anti-inflammatory drug used in this study is Indomethacin.

Results and Discussions

Results of the Antimicrobial Activities of the Ethanol extract of Scent leaf and Curry leaf

The results obtained from the antimicrobial activities of Scent Leaf (*Ocimum gratissimum L.*) and Curry Leaf (*Murrayakoenigii*) are as presented below;

Table 1: Results of the Antimicrobial activities of ethanol extract of Scent leaf (*Ocimum gratissimum L.*)

Concentration (mg/ml)	Staphylococcus aureus	Bacillus subtilis	Escherichia coli	Salmonella typhi	Candida albicans	Aspergillus niger
1.0	-	-	-	-	-	-
0.9	+	-	+	-	+	+
0.8	+	+	+	+	+	+
0.7	+	+	+	+	+	+
0.6	+	+	+	+	+	+
0.5	+	+	+	+	+	+
0.4	+	+	+	+	+	+
0.3	+	+	+	+	+	+
0.2	+	+	+	+	+	+
0.1	+	+	+	+	+	+
Ciprofloxacin 30µg/ml	-	-	-	-	+	+
Fluconazole 30µg/ml	+	+	+	+	-	-

Key:

The sign (-) indicates that there is inhibition

The sign (+) indicates that there is no inhibition

Table 2: Results of the Antimicrobial activities of ethanol extract Curry leaf (*Murrayakoenigii*)

Concentration (mg/ml)	Staphylococcus aureus	Bacillus subtilis	Escherichia coli	Salmonella typhi	Candida albicans	Aspergillus niger
1.0	+	-	-	+	-	-
0.9	+	-	+	+	-	+
0.8	+	+	+	+	-	+
0.7	+	+	+	+	+	+
0.6	+	+	+	+	+	+
0.5	+	+	+	+	+	+
0.4	+	+	+	+	+	+
0.3	+	+	+	+	+	+
0.2	+	+	+	+	+	+
0.1	+	+	+	+	+	+
Ciprofloxacin 30µg/ml	-	-	-	-	+	+
Fluconazole 30µg/ml	+	+	+	+	-	-

Key:

The sign (-) indicates that there is inhibition

The sign (+) indicates that there is no inhibition

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the extract inhibiting the visible growth of any micro-organism permanently. It is best determined by incorporating various concentrations of the extracts into the culture media, observing and measuring the zone of inhibition.

The minimum inhibitory concentration of the two leaf extracts on the microorganisms is given in the tables below:

Table 3: Minimum Inhibitory Concentration of Ethanolic extract of Scent leaf on the test micro-organisms

Test Micro-organism	Minimum Inhibitory Concentration (MIC) (mg/ml)
Staphylococcus aureus	1.0
Bacillus subtilis	0.9
Escherichia coli	1.0
Salmonella typhi	0.9
Candida albicans	1.0
Aspergillus niger	1.0

Table 4: Minimum Inhibitory Concentration of Ethanolic extract of Curry leaf on the test micro-organisms

Test Micro-organism	Minimum Inhibitory Concentration (MIC) (mg/ml)
Staphylococcus aureus	N/A
Bacillus subtilis	0.9
Escherichia coli	1.0
Salmonella typhi	N/A
Candida albicans	0.8
Aspergillusniger	1.0

Key: N/A in this context means no activity.

Table 5: Results of the Anti-inflammatory Activities of Solid extracts of Scent leaf and Curry leaf

PERCENTAGE INHIBITION OF OEDEMA FORMATION				
GROUPS	1h	2h	3h	4h
I	1.27	1.36	1.49	1.53
II	19.4	34.1	45.3	55.7
III	12.2	26.8	36.8	43.7
IV	17.8	29.7	39.7	51.2

Note: h: in the table means hour

- Group I is the toxicant control, they received carrageenan but were not treated.
- Group II is the standard control, they were treated with a standard drug, indomethacin
- Group III was treated with 100mg/kg of solid extract of Scent leaf
- Group IV was treated with 100mg/kg of solid extract of Curry leaf

Discussion of Results

Currently, in addition to antibiotics and chemically synthesized drugs, the trend to look out for alternative medicine in nature is increasing as the natural resources are less toxic and less deleterious to the overall health of human beings. In this study curry leaves and scent leaves have displayed immense potential as that natural alternative, especially with their antimicrobial anti-inflammatory activities. Tables 1 and 2 gives the antimicrobial activities of *Ocimumgratissimum L.* and *Murrayakoenigii* leaf extracts against different micro-organisms at different concentrations.

The six micro-organism used in this research were sensitive to the leaf extract of scent leaf but it showed higher activity against the bacterial strains; *Bacillus subtilis* and *Salmonella typhi* at a concentration of 0.9mg/ml (typhoid causing agent) where it showed activity even at a lower concentration. These activities was found to be more profound than the antibiotic and antifungi used in my study, alluding to the excellent medicinal properties of scent leaves without any side effects that antibiotics may produce in humans, so they can be employed for treating typhoid and other bacterial infections. There was no significant effect on other bacteria and fungi. The antimicrobial effect on the later bacteria and fungi species could have been limited by contamination in the laboratory procedures. The observation suggests that the ethanolic extracts of Scent leaf has a

higher antibacterial activity than antifungal activity as can be clearly seen from table 3. which shows the minimum inhibitory concentration (MIC) of Scent leaf.

On the other hand, not all of the six micro-organisms showed sensitivity to the ethanolic leaf extracts of curry leaves. It had more activity on the bacterial strain; *Bacillus subtilis* at a concentration of 0.9 mg/ml and a more profound activity on the fungal specie; *Candida albicans* at a concentration of 0.8mg/ml, it showed no activity on two bacterial strains; *Staphylococcus aureus* and *Salmonella typhi* as can be seen above. These limitations in activities could have been due to contamination in the laboratory procedures. It showed sensitivity on the two fungal strains used, this observation suggests that the ethanolic extracts of curry leaf has a higher antifungal activity than antibacterial activities as can be seen clearly from table 4 above which shows the minimum inhibitory concentration (MIC) of Curry leaf. This indicates that it can serve as a good alternative for treating any form of fungi and bacteria infections with little or no side effects as compared to the usual standard drugs.

Generally, from this study it can be seen that the ethanolic leaf extracts of Scent leaf and Curry leaf have very good antimicrobial activities against microbes and can be employed in place of antibiotics and antifungi for the treatment of drug resistant pathogenic strains that do not respond to the usual line of treatment. It can also be deduced from this study that the ethanolic leaf extracts of scent leaves showed a higher antibacterial activity than that of curry leaves which showed a higher antifungal activity when compared.

The standard antibiotics used, Ciprofloxacin was effective against all the bacterial strains at standard concentration (30µg/ml), while the antifungi Fluconazole was effective against all the fungal species at a standard concentration (30µg/ml).

For the anti-inflammatory activities of curry leaf and scent leaf, in this work it was observed that after the drugs were administered to the groups, they had different but similar activities. After the measurement of the paw oedema volume using the plethysmometer in 1h, 2h, 3h and 4h intervals and was used to calculate the percentage inhibition, it was observed that the solid extract of scent leaf and curry leaf showed immense activities on the paw oedema. Curry leaf (Group IV) showed a higher activity on the inflammation as its activity was closer to that of the standard drug indomethacin at the 1h, 2h, 3h and 4h intervals there was no much difference compared to the activity of Scent leaf (Group III) at this intervals, this is well illustrated in table 5.

Conclusion

Murrayakoenigii (Curry leaves) extracts have demonstrated antibacterial effects particularly on *Bacillus subtilis* and *Escherichia coli*, it has also shown antifungal effects particularly on *Candida albicans* and mildly on *Aspergillus niger* as compared to the antifungi fluconazole used in this study. The ethanolic extracts of curry leaves were found to be effective against all tested microorganism except *Staphylococcus aureus* and *Salmonelatyphi*.

For *Ocimumgratissimum L.* (Scent leaves), the results of these particular study suggests that its extracts demonstrated antibacterial effects strongly on *Bacillus subtilis* and *Salmonelatyphi*, and mildly on *Staphylococcus aureus* and *Escherichia coli*, it also showed moderate antifungal effects on *Candida albicans* and *Aspergillusniger* as compared to the antifungi used in this study. The ethanolic extracts of Scent leaves were found to be effective against all tested microorganism.

The solid extracts of Scent leaf and Curry leaf showed high anti-inflammatory activities especially that of curry leaf (Group IV) which showed an activity that is almost equal to that of the standard drug used in this study (indomethacin). Therefore the solid extracts of scent leaf and curry leaf can be comfortably used as an alternative drug to address cases of inflammation, due to their less toxicity, less side effects and high activities against any form of inflammation.

Murrayakoenigii and *Ocimumgratissimum*L has the bioactive potential and they may be the best natural alternative to antibiotic and anti-inflammatory therapy for tested microbes and paw oedema. Therefore, curry leaves and Scent leaves could be effectively used as a natural remedy in everyday meal, for the prevention of bacterial, fungal and inflammatory infections. Indeed these phenomenal plants may serve as a useful resource in the food industry and clinical medicine. This study gives way for further attention and research to identify the active compounds responsible for these plants biological activities. Also this work provides excellent insight into the anti-microbial and anti-inflammatory properties of the two leaf extracts. However, further experiments would be necessary to prove lack of toxicity of the leaf extracts at doses which would be necessary to inhibit microbial growth or exhibit anti-inflammatory properties.

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