Phytotoxic and Anti-microbial activities of Flavonoids in *Ocimum gratissimum*

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Abstract

In this study, the leaves of *Ocimum gratissimum* have been investigated and found to contain flavonoids as part of their secondary metabolites. This observation agrees with the few available reports on the presence of flavonoids in *O.gratissimum* plants. The flavonoids were extracted from dried powdered leaves of *Ocimum gratissimum* using soxhlet extraction method. The crude extract was partially purified on column chromatography using an eluting system of formic acid and ethyl acetate in the ratio of 15:85.Qualitative tests were carried out to confirm the presence flavonoids in the O. gratissimum extract. Frothing test, as a follow up was also done on the extract to ensure the absence of saponins which are usually abundant as secondary metabolites in plants. The allelopatic investigation of the partially purified extract on bean and maize seeds germination, and on seedlings growth showed that *O.gratissimum* flavonoids are phytotoxic. The inhibition of the radicle and coleoptile growth was observed to be dose-dependent, and the radicles of both seeds were comparatively more inhibited. Eight human pathogenic microbes; six bacteria and two fungi were used to evaluate the antimicrobial activities of the flavonoid extract. A broad-spectrum antimicrobial effect was observed with the flavonoids. However, they had no effect on fungi growth. [Life Science Journal 2010;7(3):45-48]. (ISSN: 1097-8135).

Key words: *Ocimum gratissimum*, flavonoids, allelopatic, phytotoxic, pathogenic microbes, antimicrobial.

INTRODUCTION

Flavoniods, as established natural anti-oxidant, have captured a fast growing interest among consumers and scientists in medical, pharmaceutical, chemical and agricultural industries. They are a group of polyphenolic compounds diverse in chemical structure and characteristics, found ubiquitously as secondary metabolites or chemical constituents in plants either consumed as foods or used by man in folk-lore medicine. They occur naturally in fruit, vegetables, nuts, grains, seeds, flowers, roots, stems and bark of plants; and are integral part of the human diet (Haslam, 1998, Middleton and Kandaswami, 1993).

Literature reports on the presence of flavonoids and other active chemical substances such as tarnins, terpenes, saponins, xanthones and glycosides as phytochemicals in different tropical plants which serve basically as foods and medicinal herbs abound and are daily on the increase following continuous scientific investigations (Sofowara, 1993). The phytochemicals present in these plants are largely responsible for the medicinal functions associated with them.

However, reports on the presence of flavonoids in *Ocimum gratissimum* are quite scanty. The genus, *Ocimum* with the general name Basil, belongs to the family of plants known as Labiatae. It is a relatively small genus of herbs and semi-woody shrubs. It is popularly called scent leaf because of its characteristic aromatic smell. About thirty species have been reported in tropical and sub- tropical parts of the word (Burkhill, 1985). *Ocimum gratissimum* is a vegetable plant of wide nutritional and medicinal applications in Nigeria and in some other parts of the world. It is therefore expedient to evaluate the bioactivities which underline the nutritional and medicinal relevance of this plant. Consequently, the objective of this work was to investigate and evaluate the plant flavonoids for its phytotoxic and anti-microbial activities.

Materials and Methods

The plant material

Fresh *Ocimum gratissimum* leaves were collected from a vegetable garden in Benin City, Edo State Southern Nigeria. The botanical identification was confirmed at the Herbarium of the Department of Botany, University of Ibadan.

Extraction of flavonoids from the plant material

The leaves of *Ocimum gratissimum* were thoroughly air-dried and were ground into powdery form using a warring blender. Five hundred grammes (500g) of the powdered leaves were put into extraction thimbles and top covered

with cotton plug. These were then extracted with absolute methanol for about 24hrs in a soxhlet apparatus to remove flavonoids and other low molecular weight compounds such as sugars, phenols, saponins and oligosaccharides (Fenwick *et al*, 1992). Prior to this, a preliminary extraction with boiling petroleum ether was carried out for 24hrs to remove lipids and various pigments. The resultant methanolic extract was evaporated to dryness in a pre-weighed beaker and its weight was determined. This extract was observed to be dark-brown in color and aromatic in flavor.

Purification of the crude methanolic flavonoid extract

The crude extract was purified with column chromatography using 250g of silica gel (F_{259} grade), a glass column size of 400mm by 3.5mm and 15:85 mixture of formic acid and ethyl acetate as eluting solvent. The eluent was collected in fractions in test tubes at a constant volume of 20ml per tube. Fifty-four fractions labeled T_1 to T_{54} were further purified with Thin-Layer Chromatography. Aliquots from each of the fifty-four fractions were collected and spotted on TLC plates using capillary tubes. The TLC plates were subsequently developed using a solvent system of formic acid and ethyl acetate in the ratio of 15:85. The developed plates were air-dried for about 30mins, and were then visualized under the UV-spectrophtometer for possible fluorescence at a wavelength of 365nm, characteristic of flavonoid spots.

Test for flavonoids in extract fractions

Two methods were used to determine the presence of flavonoids in the extract (Sofowara, 1993;Harbrone, 1973). 5 ml of dilute ammonia solution were added to a portion of each extract fraction followed by addition of concentrated H_2S0_4. A yellow coloration observed indicated the presence of flavonoids. The yellow coloration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each fraction. A yellow coloration was observed indicating the presence of flavonoid

Collection of flavonoids

The extract fractions which tested positive to flavonoid test were pooled together in a pre-weighed beaker and then concentrated. The beaker with its content was weighed after evaporation, and the difference gave the approximate weight of the partially purified flavonoids. The concentrate was dark brown slurry. It was on this that the various bioassays were carried out.

Phytotoxic Assay

1% Flavonoids solution was prepared by dissolving 1.0g of the partially purified extract in 100ml of distilled water. Serial dilutions of 0.5%, 0.25% and 0.125% flavonoid solutions were subsequently made.10 sterilized Petri-dishes were divided into two groups of five Petri-dishes for the cultivation of the bean and maize seeds. Clean cotton wool was placed at the bottom of each petri-dish, and those with bean were labeled as group I and those with maize seeds as group II.

Group I: 10mls of each of the Flavonoid solution (1%, 0.5%, 0.25% and 0.125%) were pipette into four correspondingly labeled Petri-dishes so that the cotton wool was well soaked. 10ml of distilled water was introduced into the fifth Petri-dish labeled as control. Four viable bean seeds were then arranged in a circular pattern on each of the five petri-dishes. 10ml of distilled water was added to each petri-dish everyday from the second day of the experiment. This was to make up for water loss through evaporation. The germination and growth pattern were observed for seven days. After this, the root and shoot lengths were measured using a thread and a ruler. The recorded values were compared with those of the control groups to determine the index of inhibition of seed germination and growth. The same procedure was concurrently used for the maize seeds and labeled as group II.

Antimicrobial assay

20 mg/ml flavonoid solution was obtained by dissolving 0.2g of the extract in 10ml of sterile distilled water, and used as the standard extract concentration (*Hirasawa et al, 1999*). 10mg/ml, 5mg/ml, and 2.5mg/ml test solutions were subsequently prepared by serial dilution, using water as the diluents.The culture media used were carefully handled and prepared according to the manufacturer's instruction. They were all commercial products of oxoid Ltd Company, England. Six bacteria (*Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeriginosa, Proteus mirabilis, Klebsiella spp)* and two fungi (*Candida albicans and Aspergillus niger*) were used for this assay. The antibacterial and antifungal activities of the test sample were done using the agar well diffusion method (Stoke and Ridgeway, 1980). Sterile distilled water used as the control. The inhibitory zones produced were measured in millimeters. Negative results were regarded as those in which no zone of inhibition was observed.

RESULTS AND DISCUSSION

The results of the effect of flavonoids on the growth profile of maize and bean seedlings are presented in Table 1. Significant difference between the shoot/root lengths of the treated bean/maize and control seedlings was observed mainly at flavonoid concentrations of 0.5% and 1.0%. The germination of seeds occurred faster in the control media relative to those treated with flavonoid extract, meaning that the flavonoids from *O.gratissimum* inhibit seed germination. Generally, the bean seeds germinated faster than their maize counterparts. This indicates that the maize seeds were more susceptible to the phytotoxic effects of the Flavonoids. Moreover, the inhibitory effects on the germination of bean and maize seeds and on their radicules and coleoptiles were observed to increase with increase in the concentration of the extract. This implies that the inhibitory activity of the *O.gratissimum* flavonoids is dose dependent.

Tables 1a & 1b show that O.gratissimum flavonoids are phytotoxic even at low concentrations. At concentrations of 0.1%, 0.25%, 0.5% and 1.0% the *O,gratissimum* flavonoids in a dose-dependent pattern reduced the growth profile of both maize and bean seedlings. There was a high indication

that these Flavonoids retarded seed germination and seedling growth by inhibiting certain endogenous growth hormones such as gibberellic acid (GA3), indol acetic acid oxidase (IAA-oxidase) and indo-3-acetic acid (IAA) [responsible for seedling elongation]. Thus, their application in agriculture could be considered in weed control

The *O.gratissimum* flavonoids were effective against all bacteria used, both gram positive and gram negative at dose concentrations of 20mg/ml and 10mg/ml. At 5mg/ml, the flavonoids were only effective against *E.coli* and *Proteus mirabilis* and at 2.5 mg/ml, *E.coli* was the only microbe susceptible to the antimicrobial effect of O.gratissimum flavonoids.(Table 2c).This indicates that *O.gratissimum* flavonoids have a broad-spectrum anti-bacterial activity. They are however not antifungal. The inhibitory effect of the flavonoid extract on the selected bacteria was observed to increase with increased concentration of the flavonoid extract, indicating a dose dependent effect. At 20mg/ml dose concentration, flavonoids in *O.gratissimum* showed a potency range of 58.3% to 85.7% relatively to the various standard antibiotics used as positive control (Table 2c). The antibiotics used as standard (positive control) against the pathogenic organisms are standard orthodox drugs used for the treatment of infections in which the pathogens are implicated (Table 2a). Moreover, the bacteria against which the *O.gratissimum* flavonoids were effective are pathogens already implicated in the etiology and severity of human diseases. Thus, these flavonoids may probably be of immense potential application in pharmaceutical and medical formulations. The possibility of further purification and formulation of these flavonoids into antibiotics should be considered.

Table 1a: Effect of *O.gratissimum* Flavonoids on the growth profile of bean and maize seedlings.

Conc. of Flavonoid extract	ASL (cm), Beans	ASL (cm), Maize	ARL (cm), Beans	ARL(cm), Maize
0.10%	8.02	7.45	5.87	5.22
0.25%	7.01 [b]	6.77	5.12 [b]	4.53
0.50%	6.08 [b]	5.81 [b]	4.01 [a]	3.84 [b]
1.00%	4.06 [a]	3.98 [a]	2.52 [a]	2.41 [a]
Control	8.86	7.91	7.20	5.93
a= significant difference ($p<0.05$) against control	b= significant difference ($p<0.01$) against control			

ASL= Average shoot length, ARL= Average root length. *Values are average results of six sets of the experiment*

Table 1b: Percentage inhibition of root/shoot growth profile by O.gratissimum fllavonoids.

Conc. of Flavonoid extract	% inhibition of shoot, Beans	% inhibition of shoot, Maize	% inhibition of root, Beans	% inhibition of root, Maize
0.1%	9.48	5.80	18.47	11.17
0.25%	20.88	14.4	28.89	23.60
0.5%	31.38	26.50	44.31	34.73
1.0%	54.17	49.70	65.00	59.36

Table 2a: Pathogenic organisms and selected standard antibiotics (+ve control)

Pathogenic organism	*S. aureus*	*E. coli*	*B. subtilis*	*P. aeruginosa*	*P.mirabilis*	*Klebsiella spp.*	*A. niger*	*C.albicans*
Standard antibiotic	Gentamycin			Tetracycline			Nystatin	

Table 2b: Diameters (mm) of inhibition zones of microbial growth by O.gratissimum flavonoid extract

			Conc. of Flavonoid extract (mg/ml)			
Organism	**-ve control**	**+ve control**	**2.5**	**5.0**	**10.0**	**20.0**
Staphyococcus aureus.	0	22	0	10	14	18
Escherichia coli	0	28	12	15	18	24

Bacillus subtilis	0	20	0	0	10	14
Pseudomonas aeruoginosa	0	18	0	0	10	12
Proteus mirabilis	0	24	0	28	12	16
Klebsiella spp.	0	23	0	0	12	15
Aspergillus niger	0	22	0	0	0	0
Candida albicans	0	16	0	0	0	0

Values are mean results of six sets of the experiment

Table 2c: Percentage (%) inhibition of microbial growth by O.gratissimum extract

	Conc. of Flavonoid extract (mg/ml)			
Organism	**2.5**	**5.0**	**10.0**	**20.0**
Staphyococcus aureus.	0	45.5	63.6	81.8
Escherichia coli	42.9	53.6	64.3	85.7
Bacillus subtilis	0	0	50.0	70.0
Pseudomonas aeroginosa	0	0	55.6	66.7
Proteus mirabilis	0	30	50.0	58.3
Klebsiella spp.	0	0	52.0	65.2
Aspergillus niger	0	0	0	0
Candida albicans	0	0	0	0

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