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Studies on the germination, chemical composition and antimicrobial properties of *Cucumis metuliferus*

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ABSTRACT

Studies on the germination, chemical composition and antimicrobial properties of Cucumis metuliferus were investigated. Soaking and temperature regimes showed a significant ($P<0.05$) effect on the germination of C. metuliferus seeds. The cumulative germination percentage was comparatively higher in the control with 96.66%, while seeds soaked for 12 and 36 hours had 93.34 and 83.31% respectively. Temperature regimes and period of exposure affected germination and seeds exposed to 60^0C for 30 minutes had 90% germination while, seeds exposed to 120^0C for 60 minutes had 60% germination. Phytochemical screening revealed the presence of alkaloids, flavonoids, triterpenoid, saponins, steroids, volatile oils, total glycosides, cardiac glycosides and saponin glycosides. Proximate and mineral analysis of both leaves and fruits revealed that it contain substantial amount of nutrients evaluated. The antimicrobial properties of C. metuliferus fruits were investigated using acetone, methanol and water extracts against nine organisms (four bacteria species: Escherichia coli, Bacillus subtilis, Pseudomonas aureus and P. aeruginosa and five fungal species: Aspergillus niger, A. flavus, Fusarium solani, Trichophyton mentagrophyte and Microsporum canis). The extract has no bactericidal effect on the species tested but showed significant effect on the fungal species tested except A. niger at 5 mg ml^{-1}. The results of this study indicates that soaking promotes early seed germination which could reduce the risk of attack by soil microbes or lack of sufficient moisture and C. metuliferus contain nutrient and substantial amount of secondary metabolite which reinforced its use as vegetable and in medicine.

Keywords: germination, temperature, antimicrobial, secondary metabolites, *Cucumis metuliferus*

INTRODUCTION

Recently, the quest for the use of plant materials for medicaments as against the use of orthodox medicine has gained interest in the field of science. The usefulness of plant materials in the treatment of diseases has been demonstrated to be as a result of the presence of certain chemical compounds in plants, which include flavonoids, alkaloids, steroids, tannins and saponins [8]. Phytochemical compounds are non specific in their action and can exhibit several functions viz antibacterial [7], anti-fungal [13] antiviral and anti-spasmodic [9].

Cucumis metuliferus commonly called African horned cucumber belongs to the family cucurbitaceae, and is a monoecious, climbing and annual herb that can be grown practically anywhere provided the season is warm [2]. The fruits are ovoid berries of 8-10 cm long and 4-5 cm in diameter, reddish orange at maturity covered with strong spiny outgrowths. The seeds are embedded in the mesocarp which is emerald green and consist of juicy bland tasting tissues. The fruits occur in two forms; the bitter form contains cucurbitacins (triterpenoids) which is a highly toxic

compound and the non bitter form has been found to be less toxic and hence widely cultivated [17]. The fruit extracts could be beneficial in increasing sperm integrity [12] and the alkaloid component of its fruit is a potent antiviral agent since it prevented and reversed virus induced manifestation in infected chicks [11].

High resistance of microorganism to most conventional antibiotics coupled with the exorbitant cost of medicine in developing countries necessitates the search for cheaper and safer alternative drugs available to common man. African horned cucumber has been claimed to be useful in treatment of various ailments, but most of this claims have not been scientifically validated. In the current study, germination studies of its seeds, phytochemical analysis and antimicrobial activity of *C. metuliferus* were investigated.

MATERIALS AND METHODS

The fruits, seeds and leaves of *C. metuliferus* were collected from a garden located at No. 7 Zuru Road, Sokoto. The fruits and leaves were air dried and pulverized into powdered. For germin ation studies, group of 50 seeds were exposed to varied temperature regimes of 60, 80, 100 and 120^{0}C each for periods of exposure of 30, 45 and 60 minutes. Also effect of soaking period on germination of *C. metuliferus* was determined using 0, 12, 24 and 36 hours of soaking. After these treatments (temperature and soaking), the seeds were placed in petri dishes containing one layer of 9 cm Whatmann filter paper which was moistened with distilled water to ensure adequate moisture for the seeds. The petri dishes containing the seeds were then left on the laboratory bench and observed at 37$\pm$ 2^{0}C. Seeds were considered to have germinated when the ridicule had grown to 2 mm [15]. The number of germinated seeds was recorded daily for 15 days.

The grounded material (fruits and leaves) of C. metuliferus was subjected to phytochemical screening test, proximate and nutrient analysis. Alkaloids, tannins, saponins, flavonoids, total glycosides, triterpenoids, steroids, anthraquinone, volatile oil and balsam were determined using the method of to the method of Trease and Evans [6] and El Olemy *et al*. [10]. Ash content, total lipids, crude fibre and carbohydrate estimation were determined using AOAC methods [1], while crude protein was determined using Macro kjeldahl. Sodium and potassium were determined by flame photometry method while calcium and magnesium were determined using EDTA titration method.

Antimicrobial studies (bacterial and fungal sensitivity testing) were carried out using varied concentration (5, 10, 20, 40 and 80 mg/ml) of water, acetone and methanol extracts of grounded fruits and leaves of *C. metuliferus*. The test organisms were obtained from microbiology research laboratory and mycology laboratory both in Usmanu Danfodiyo University, Sokoto. Four (4) bacterial species (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*) and five fungal species (*Aspergillus flavus, A. niger, Fusarium solani, Trichophyton mentagrophyte* and *Microsporum canis*) were used for the study.

Bacterial sensitivity test was carried out using disc diffusion method; a paper disc of 5 mm was made from Whatmann filter paper, counted and put into a test tubes containing varied extract concentrations already prepared. This was allowed to absorb the extract for 24 hrs before the test organisms were inoculated into the nutrient agar media plates by means of sterile wire loop. The plates were incubated at 37^{0}C for 24 hrs. The diameter of the zone of inhibition was measured in millimeter. For fungal sensitivity testing, agar incorporation method was used; sterile syringe was used to measure 5mls of the prepared varied extracts solutions and mixed with the prepared PDA media. The test organisms collected were inoculated on the plates using 2 mm cork borer. The mixture was poured on the plates after being shaken gently. The plates were incubated at room temperature for the period of five (5) days. A meter rule was used to measure the linear dimension of growth (in mm). The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{Control value} - \text{Treated samples}}{\text{Control}} \times 100$$

The data obtained were subjected to analysis of variance (ANOVA) and differences between means of treatments were calculated using least significance difference (LSD) test at 5% level of probability.

RESULTS AND DISCUSSION

Germination Studies

The effect of soaking showed a significant effect (P<0.05) on the seed germination of *C. metuliferus* (Table 1). It was observed that soaking promoted earlier germination than the control seeds. However, high percentage germination was recorded in the control more than the soaked seed. Seeds soaked for 12, 24 and 36 hours germinated one day earlier than the control. The germination of untreated seeds (control) was completed within a maximum of eleven days, but the treated seeds for 12 and 36 hours completed in ten and thirteen days respectively. Untreated seeds had the highest seed germination of 96.66%, followed by those soaked for 12 and 36 hours with 93.34 and 83.31% respectively. Prolonging soaking period for 24 and 36 hours affected the seed germination of C. *metuliferus* negatively. Although, soaking enhanced earlier seed germination as compared with the control (untreated seed), but high seed germination was observed. Water is an important factor in the germination of seed and it soften the seed coats and enable the radicle and epicotly to break through them more easily. It also promotes the entrance of oxygen into seed, for gases pass more readily through moist cell walls than through dry walls. But high water content of seed reduced the oxygen availability which in turns slow down germination process. This may however be the case as observed from the result obtained in this study (Table 1). A similar observation was reported by Sabongari and Aliero [16] on the germination of tomato (*Lycopersion esculentum*) seeds.

The result of the effect of temperature on the daily seed germination of *C. metuliferus* is presented in Table 2. Also, table 3 presented a significant effect (P < 0.05) on the mean percentage germination of *C. metuliferus* as affected by temperature regimes and periods of exposure. Seed exposed to 60^0C for 30 minutes recorded high percentage germination of 90% while exposure to 100^0C for 30 and 45 minutes both recorded percentage germination of 87%. It was observed that exposure to 120^0C resulted in low germination. It was also observed that the seed germination decreased with the increased in temperature. Treatment time was observed to have exerted a significant effect on the seed germination. Based on the results obtained in this study, it was observed that C. *metuliferus* has high seed viability as supported by its high percentage germination count of over 75%. According to John and Paul [5], high percentage count is considered as the basis for determining viability of the seeds. High temperature affects plant regrowth potential and seed germination [4]. The temperature requirements for the germination of seed usually coincide with temperature requirement for the growth of active plant organs. Seeds of different species vary widely in their temperature requirements for germination. Result obtained in this study (Table 2) indicated that the optimum temperature needed to break the innate seed dormancy in *C. metuliferus* range from 60-100^0C within an exposure time of 30-60 minutes, but at 100^0C the exposure time fall within the range of 30-45 minutes. Above 100^0C for 45 minutes, only short exposure time led to successful germination, while longer exposure caused seed death. Koduru *et al*. [15] showed that high temperature applied to green mature and yellow, dry seeds of *Solanum aculeastrum* break their innate dormancy and observed that above 100^0C and short exposure time led to successful germination, while longer exposure caused seed death in *S. aculeastrum*

Phytochemical and Nutrient Evaluation

Results of phytochemical screening revealed the presence of most secondary metabolites evaluated except tannin, anthraquinone and balsam in fruits and leaves (Table 4). The fruits contained large quantity of alkaloids and cardiac glycosides with moderate contents of saponin, total glycoside, volatile oils, Flavonoids and triterpenoids. The leaves contain moderate content of alkaloids and saponin while other components are trace quantity. In this study, the high content of alkaloid suggests high toxicity and medicinal property which may be responsible for its antimicrobial activity. Similar trend was reported by Idowu *et al.* [18] on antimicrobial potentials of *Cryosophyllum albidum* seeds. The large quantity of cardiac glycoside obtained from this study suggests that the plant may cause weakness, fatigue, depression and confusion if consumed.

The result of proximate analysis of *C. metuliferus* (table 5), revealed the presence of high concentration of carbohydrate 62.25 and 44.56% in fruits and leaves respectively. Crude protein content revealed 9.04 and 9.77 % in fruits and leaves respectively. The low fibre content and significant amount of crude protein indicated that the plants could be beneficial in fattening programmes. According to Lolade [3] high level of crude protein is responsible for the conferment of transient immunity. Mineral nutrient composition of *Cucumis metuliferus* is presented in Table 5. The result revealed the presence of high concentration of potassium (180.8 and 966.3 mg/kg) for leaves and fruits respectively. Sodium content analysis revealed 161.7 and 126 mg/kg for leaves and fruits respectively. Higher content of Na and K in *C. metuliferus* fruits and leaves inferred better nutritional potentials as these elements

together with calcium may serves as activators of energy potentials across nerve membrane, replenishment in diarrheic condition, maintenance of normal nervous functions and gut peristalsis [16].

Antimicrobial activity

The result of antibacterial assay of acetone, methanol and water extracts did not show a significant effect on the growth of bacterial species tested. However, the growth of *S. aureus* was suppressed by the water extract at 90 mg/ml. The results of the antifungal activity of *C. metuliferus* extracts at various concentrations (5, 10, 20, 40 and 80 mg/ml) on the growth of certain fungal species tested is shown in Table 6. The growth of *A. niger* was inhibited by the acetone extract with inhibitory percentage of 56.44 and 81.00 % at 20 and 80 mg/ml respectively. Methanol showed inhibition of 54.80 and 55.33% at 40 and 80 mg/ml. the water showed inhibition only at 80 mg/ml (Table 6). The result of this study shows that *C. metuliferus* fruit has inhibitory effect on all the species tested except *A. niger*. The result of this study suggest that *C. metuliferus* could be used in the treatment of *Aspergillosis* diseases e.g. Asthma, Aspergilloma e.t.c caused by Aspergilla. This is due to the inhibitory effect of this plant against *A. flavus* at the concentration of 80 mg/ml. Similarly, the result suggests that *C. metuliferus* could be used in the treatment of skin diseases such as *mycosis*, *Onychomycosis*, *Tinea corporis* e.t.c which are caused by the dermatophytes (*T. mentagrophyte* and *M. canis*).

Table1: Effect of soaking period on the germination of *C. metuliferus*.

Soaking period (hours)	Germination % (days)					
	2	4	6	8	12	15
0	$33.33^{c} \pm 2.50$	$86.66^{a} \pm 1.80$	$86.66^{b} \pm 1.80$	$89.99^{a} \pm 0.40$	$96.66^{a} \pm 0.93$	$96.66^{a} \pm 0.93$
12	$53.32^{b} \pm 1.60$	$83.34^{b} \pm 2.90$	$89.99^{a} \pm 0.47$	$89.99^{a} \pm 0.47$	$93.34^{b} \pm 0.47$	$93.34^{b} \pm 0.47$
24	$59.99^{a} \pm 0.90$	$69.99^{c} \pm 0.80$	$69.99^{c} \pm 0.8$	$73.33^{b} \pm 0.40$	$76.66^{d} \pm 0.40$	$79.99^{d} \pm 0.5$
36	$33.33^{c} \pm 2.10$	$56.66^{d} \pm 0.47$	$59.99^{d} \pm 0.4$	$66.66^{c} \pm 0.50$	$79.99^{c} \pm 0.10$	$83.31^{c} \pm 0.3$
SE ±	0.62	0.20	0.26	0.28	0.24	0.24

Values represent means and standard deviation of percentage germination. Mean in a column with the same superscript are not significantly different at (P< 0.05).

Table 2: Mean daily germination percentage of *C. metuliferus* as affected by temperature and exposure time

Temperature (0^{0}C)	Period of Exposure (Min.)	Germination % (Days)					
		2	4	6	8	12	15
60	30	70	90	90	90	90	90
	45	73	80	80	80	80	80
	60	70	80	83	83	86	87
80	30	77	80	80	80	80	80
	45	70	77	80	80	80	80
	60	80	83	83	83	83	83
100	30	77	87	87	87	87	87
	45	63	87	87	87	87	87
	60	50	60	60	60	66	66
120	30	73	83	87	87	87	87
	45	33	50	60	60	60	60
	60	20	60	60	60	60	60

Values represent mean of three replicates of percentage germination.

Table 3: Mean percentage germination of *C. metuliferus* as affected by temperature and period of exposure

Treatment	Germination %
(A) Temperature	
60	85.56^{a}
80	81.11^{a}
100	82.22^{a}
120	66.67^{b}
S.E ±	4.27
(B) Exposure	
30	84.17
45	76.67
60	75.83
S.E ±	3.70

Values represent means of three replicates of percentage germination.

Table 4: Qualitative Phytochemical analysis of the secondary metabolites of fruits and leaves of *C. metuliferus*

Constituents	Plant parts	
	Fruits	Leaves
Alkaloid:	+ + +	+ +
Tannin	N. D	N.D
Saponin	+ +	+ +
Flavonoids	+ +	+
Total glycoside	+ +	+
Saponin glycoside	+	+
Triterpenoid	+ +	N.D
Cardiac glycoside	+ ++	N.D
Steroid	N.D	+
Anthraquinone	N.D	N.D
Volatile oil	+ +	+
Balsam	N.D	-

KEY: *+ =Trace amount, + += Moderate constituent,* ***+*** *++= large constituent,* ***-*** *= absent. N.D = not detected*

Table 5: Proximate (%) and mineral Composition (mg/kg) of fruits and leaves of *Cucumis metuliferus*

Nutritional Composition (%)	Fruit	Leaf
Ash	4.8 ± 2.3	1.33 ± 6.75
Lipid	7.8 ± 0.76	2.6 ± 0.30
Fiber	6.2 ± 0.76	11.66 ± 0.77
Crude Protein	9.04 ± 0.13	9.77 ± 0.30
Carbohydrate	62.25 ± 1.95	44.56 ± 0.13
Mineral elements (mg kg^{-1}):		
Sodium	161.70 ± 3.8	126 ± 6.36
Potassium	180.8 ± 130.70	966.30 ± 355.70
Calcium	0.37 ± 0.03	0.52 ± 39.0
Magnesium	0.16 ± 0.03	2.97±725.0
Phosphorus	0.75±0.016	0.58±701.4

Table 6: Inhibitory effects of different types of *C. metuliferus* extracts on some selected fungal species

Extracts	Concentration (mg/ml)	Growth inhibition (%)				
		An	Af	Fs	Tm	Mc
Acetone	5	0	0	76.70	50.20	0
	10	0	0	78.00	58.00	39.40
	20	56.44	0	79.70	62.80	51.70
	40	62.00	51.70	73.00	63.60	73.10
	80	81.00	69.80	73.11	74.10	78.10
Methanol	5	34.22	0	62.22	47.20	0
	10	47.44	56.90	62.80	49.70	34.10
	20	49.80	61.40	64.11	53.30	52.60
	40	54.80	61.70	68.70	53.60	54.40
	80	55.33	66.11	72.60	62.20	56.70
Water	5	0	0	66.70	0	56.30
	10	33.33	48.60	71.11	38.90	63.70
	20	44.70	58.33	72.44	45.30	71.90
	40	45.00	58.60	73.00	49.20	73.30
	80	51.70	60.60	73.11	83.30	78.90
Control (PDA Fulcin)	20	0	0	0	0	0
	5	80.00	83.90	84.40	82.20	79.40
	80	97.80	95.60	95.60	97.80	95.60

Values and means of percentage growth inhibition of three replicates

Keys*: An = Aspergillus niger, Af = Aspergillus flavus, Fs = Fussarium solani, Tm = Trichophyton mentagrophyte and Mc = Microsporum canis.*

CONCLUSION

The results of this study indicates that soaking promotes early seed germination which could reduce the risk of attack by soil microbes due to lack of sufficient moisture and *C. metuliferus* contained essential nutrients, antifungal potentials and substantial amount of secondary metabolite which reinforced its use as vegetable and in medicine.

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