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# Inhibitory Effect of Methanolic Extract of *Annona senegalensis* against Seed Germination and Seedling Growth of Four Selected Seeds

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**Abstract** We investigated the allelopathic activity of methanol extract of *Annona senegalensis* against four selected seeds. The percentage seed germination was evaluated for 72 hours and percentage inhibition of seedling growth was tested for 7day and 14 days respectively. The result showed that inhibition activity of the seed germination and seedling growth was in these order; amaranthus, tomato, maize and cowpea. The plant extract showed considerable allelopathic activity and the inhibition effect of the extract against the tested seeds increased with increase in concentration of the extract.

**Key words**

Allelopathic activity, *Annona senegalensis* ,Amaranthus , Tomato, Maize , Cowpea

The environmental and health hazards from the use of herbicide have led to find the alternative methods of weed management. Among such alternatives, one is the use of allelopathic crops (12). They release chemicals into the soil that can contribute to weed management through suppression of weed seed germination, seedling emergence and establishment, and seedling growth (13). Although, the main mechanism of weed dissemination is through natural propagation, however, the uncomposted crop residues and animal excreta may also cause weed infestation when applied as soil amendment (2). Biological control and integrated management of plant diseases and weeds are considered a part of sustainable farming system. Numerous crops and weeds have been investigated for their allelopathic characteristics. Grain sorghum, rye, oats, wheat, and many weed species are suspected of heterotoxicity (allelopathy from plants of different species) (12). (14) achieved 90% weed control in paddy fields by incorporating *Datura stramonium*, *Desmodium triflorum* and *Melia azedarach* each at 1 t ha-1 separately, and 70% weed reduction at 2 t ha-1 of *Clerodendrum trichotomum* biomass. The organic amendments hold great promise as a source of multiple nutrients and ability to improve soil characteristics (17), but if applied injudiciously they may also cause some tribulations. *Annona senegalensis* is a subtropical plant (25) that has been implicated for the treatment of chest pain, coughs, anaemia, urinary tract infection (5 ; 23), cancer treatment (8;11]), diarrhoea, dysentery (9: 19), anthritis and rheumatism (7;4). *A. Senegalensis* Pers (Annonaceae) commonly known as "Wild Custard Apple" is a shrub or small tree widely distributed in Africa (1; 24). In Nigeria, *A. senegalensis* is variously known as "Gwandardaji" in Hausa, "Abo" in Yoruba, "Uburuocha" in Ibo, and "Ikpokpo" among the Idoma speaking people in the Middle Belt region of Nigeria (15). The genus *Annona* is characterized by presence of acetogenins (27) alkaloids other classes of compounds including carbonhydrate, lipids, amino acids, polyphenols, and essential oils terpenoids (18).
Previous work on *A. senegalensis* has shown antidiarrheal (30), antimicrobial (22), anticancer (29), trypanocidal (24), antimalarial and cytotoxic (3), anticonvulsant (10), analgesic, anti-inflammatory (1), antiulcer/antacid, smooth muscle relaxant (20), antibacterial (21;23), antitumor (11; 27), antiprotozoal (15), molluscicidal (28) and hormone-mimetic (16) activities. The isolation of monotetrahydrofuran and bis-tetrahydrofuran acetogenins (27) and two cytotoxic monotetrahydrofuran acetogenins (26) from this plant are also documented.
This work presents the inhibitory effects of methanolic extracts of *Annona senegalensis* on seed germination and seedling growth bioassay of four selected seeds

## Materials and Methods

### Plant Sample

The plant sample was collected at Akure town in Ondo State and authenticated at Federal Research Institute of Nigeria (FRIN) in Ibadan. Aerial part of the plant was collected. This was air-dried at room temperature under the cool air away from the sun. The dried plant was pulverized and kept in an air-tight polythene bags

### Preparation of Extract

500g of dried and pulverized plant material was weighed and poured into 6ltr flat bottom flask. 1.5ltrs of N-Hexane was poured into the flask and this was covered with aluminium foil and made air-tight with Paper tape. This is in a bid to de-fat the plant material. After 24hrs, the supernatant was decanted and the plant

material air-dried again. The material was thereafter poured again into the flask 2ltr of MeOH added. This was made air-tight and left at room temperature for 72hrs. The supernatant was thereafter decanted and concentrated using a Rotary Evaporator. The yield was 16.65g (3.33%)

## Treatment

From the crude extracts, three treatments of different concentrations (0.0, 1.0, 2.5, and 5.0% w/w) were prepared in Methanol: two controls were prepared – Methanol and distilled water. The different treatments for each one of the organic extracts were obtained from a stock solution which had been previously prepared from each of the raw extracts. The concentrations were 0.0, 1.0, 2.5 and 5.0% v/v for the methanolic extract

## *In vitro* biotest

## Seed germination test

The test was carried out according to the method of Casimiro *et al*., 2017 with slight modification. Viable seeds were obtained from Agricultural Development Parastatal (ADP) in Akure, Ondo state. Concentration of 5 %, 2.5 % and 1 % (w/v) of the crude extract were used to treat the filter papers placed inside Petri dishes 10 cm in diameter and air-dried at room temperature. 10 seeds per treatment were placed into the each Petri dish. Two control set-ups were prepared in a similar way with pure solvent and distilled water, allowing each to also evaporate fully. The experiment was carried out with three repetitions per treatment. The Petri dishes were placed in a dark cupboard with relative humidity and room temperature. The evaluation of germination was performed 72 h after the introduction of seeds. Germination percentage (G %) was calculated by dividing the total number of seeds that germinated on after 72 h in each treatment by the number of seeds sown and multiplied by 100. The percentages of germination inhibition were calculated by comparison with the untreated control, using the following calculation: % inhibition = $(C - X)/C \times 100$, where $C$ is the number of seeds germinated in control and $X$ is the number of seeds germinated in the test sample.

## Seedling Growth Test:

The test was carried out according to the method of Casimiro *et al*., 2017 with slight modification. Viable seeds were obtained from Agricultural Development Project (ADP) in Akure, Ondo state. Concentration of 5 %, 2.5 % and 1 % (w/v) of the crude extract were used to treat the filter papers placed inside Petri dishes 10 cm in diameter and air-dried at room temperature. 10 seeds per treatment were placed into the each petri dish. Two control set-ups were prepared in a similar way with pure solvent and distilled water, allowing each to also evaporate fully. The experiment was carried out with three repetitions per treatment. The petri dishes were placed in a dark cupboard with relative humidity and room temperature. Seedling growth was evaluated for 7 days and 14 days after the introduction of the seed. The percentages of inhibition were calculated by comparison with the untreated control, using the following formula: % inhibition = $(C - X)/C \times 100$, where $C$ is the average length of shoot/root in control and $X$ is the average length of the shoot/roots in the test sample

## Result and Discussions

## Percentage Seed Germination

The result of the percentage seed germination is presented in Table 1. Tomato had the highest percentage seed germination of 23.33%, followed by maize (20.00%), cowpea (13.33%) and amaranthus had no percentage seed germination for 5% (w/v) concentration of the methanolic extract of *Annona senegalensis*. For 2.5% (w/v) concentration, the percentage seed germination was found to be in the order: tomato (33.33%), maize (26.67%), cowpea (20.00%) and amaranthus (6.67%). For 1% (w/v) concentration of the extract, percentage seed germination was found to be in the order: tomato (40.00%), maize (40.00%0, cowpea (33.33) and amaranthus (10.00%). From the study, it showed that the higher the concentration of the extract, the lower the percentage of seed germination. This finding was in agreement with the work of Ines *et al*., (2014) who reported that the percentage seed germination of *Lactuca sativa* in the presence of aqueous extract at difference concentration of Tunisian and Indian varieties of *Nigella sitiva* seeds and aerial parts harvested at vegetative, flowering, and fruiting stage, decrease with increase in the concentration of the extract.

Table 1

**Percentage seed germination of methanolic extract of *Annona senegalensis* on four selected seeds.**

| concentration | Amaranthus | Cowpea | tomato | Maize |
|---|---|---|---|---|
| %5 | $0.00^{a}$±0.00 | $13.33^{a}$±6.67 | $23.33^{a}$±3.33 | $20.00^{a}$±0.00 |
| 2.5% | $6.67^{a}$±3.33 | $20.00^{ab}$±0.00 | 33.33a$^{b}$±3.33 | $26.67^{ab}$±3.33 |
| 1% | $10.00^{b}$±0.00 | $33.33^{b}$±3.3 | $40.00^{b}$±0.00 | $40.00^{b}$±0.00 |
| Control (MEOH) | $100.00^{c}$±0.00 | $66.67^{c}$±3.33 | $96.67^{c}$±3.33 | $76.67^{c}$±3.33 |
| Control (withoutMEOH) | $100.00^{c}$±0.00 | $76.67^{c}$±3.33 | $100.00^{c}$±0.00 | $80.00^{c}$±5.77 |

Values are means of triplicate ± standard error. Column means followed by the same superscript letters are not significantly different at P<0.05.

## Percentage inhibition of seed germination

The result of percentage inhibition of seed germination is presented in Table 2. The result showed that the higher the concentration, the higher the inhibition percentage of the seed germination. For 5% (w/v), the inhibition percentage was in these order; amaranthus (100.00%), maize (73.81%), tomato (72.22%) and cowpea (68.05%). For 2.5% (w/v) concentration of the extract, the inhibition percentage was in these order; amaranthus (93.33%), tomato (65.18%), maize (64.88%) and cowpea (59.31). for 1% (w/v) concentration of the extract, the percentage inhibition was in these order; amaranthus (90.00%), tomato (58.55%), maize (47.62%) and cowpea cowpea (45.33%). The overall inhibition effect of the extract against the seeds were in these order; Amaranthus, tomato, maize and cowpea. this result also showed should that the higher the concentration of the extract, the higher the concentration of the percentage inhibition of the seed germination and these findings agreed with the previous work of Casimiro *et al*., (2017) who reported that the allelopathic activity of ethanolic extract of *Arachis hypogaea* on the growth of hypocotyl and rootlet of *L.sativa* increased with increase in the concentration of the extract.

Table 2

**Percentage inhibition of methanolic extract of *Annona senegalensis* against four selected seeds**

| Concentration | Amaranthus | cowpea | tomato | Maize |
|---|---|---|---|---|
| 5% | $100.00^{c}$±0.00 | $68.05^{b}$±16.02 | $72.22^{b}$±4.00 | $73.81^{c}$±1.19 |
| 2.5% | $93.33\pm^{bc}$3.33 | $59.31^{b}$±3.77 | $65.18^{b}$±4.82 | $64.88^{c}$±5.29 |
| 1% | $90.00^{b}$±0.00 | $45.23^{ab}$±8.58 | $58.55^{b}$±1.44 | 47.62b±2.38 |
| Control | $0.00^{a}$±0.00 | $8.33^{a}$±4.17 | $3.33^{a}$±0.03 | $3.70^{a}$±0.37 |

Values are means of triplicate ± standard error. Column means followed by the same superscript letters are not significantly different at P<0.05.

## Seedling growth bioassay

The result of the percentage inhibition of the seedling growth is presented in Table 3. For 5% (w/v) and 2.5% (w/v) concentration of the extract, the inhibition activity were higher at $7^{th}$ day than the 14th day except for the cowpea and maize where the inhibition activity was higher at $14^{th}$ day than $7^{th}$. For 1% (w/v) concentration of the extract, inhibition activity of extract was higher at $14^{th}$ day than $7^{th}$ day against the tested seeds. The result showed that in most cases the inhibition *N. sitava* activity against root of the seeds were higher than their corresponding shoot and these was in agreement with the previous by Ines *et al*., (2014) who reported that the inhibition index of the aqueous extract of Tunisian and Indian varieties of seed and aerial parts against *L.sativa* germination growth had higher value in root length than the shoot length and that half inhibition concentration (IC50) of root growth was lesser than shoot growth. The result was also in agreement with the work of Casimiro *et al*., (2017) who reported that inhibition activity of *Arachis hypogaea* was higher against rootlet of L. sativa than the hypocotyl.

## Conclusions

The result from this research had showed that methanolic extract of A*nnona senegalensis* had allelopathic potential against tested seed, therefore further studies on isolation and charactertization of bioactive components in the extract that may be responsible for the allelopathic activity should be carried out.

Table 3

**Percentage inhibition of methanolic extract of *Annonasenegalensis* against seedling growth of the four selected seeds**

| Conc | Tomato | | | | Amaranthus | | | | cowpea | | | | maize | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 7 days | | 14days | | 7days | | 14days | | 7days | | 14days | | 7days | | 14day | |
| | Shoot | root | Shoot | Root | shoot | Root | Shoot | root | shoot | root | shoot | root | shoot | root | shoot | root |
| 5% | $70.68^{c}$±8.25 | $86.74^{c}$±0.96 | $57.25^{c}$±5.10 | $76.51^{c}$±4.10 | $89.70^{c}$±5.69 | $89.62^{b}$±6.23 | $63.22^{c}$±7.36 | $75.08^{b}$±5.73 | $33.13^{b}$±2.79 | $38.42^{c}$±2.69 | $56.52^{d}$±1.91 | $64.74^{b}$±2.44 | $46.40^{c}$±5.80 | $20.97^{c}$±2.72 | $46.57^{d}$±1.86 | $62.89^{b}$±3.22 |
| 2.5% | $49.08^{b}$±2.99 | $72.48^{c}$±3.32 | $54.57^{c}$±4.22 | $66.25^{bc}$±2.72 | $80.96^{c}$±9.75 | $82.44^{b}$±6.23 | $55.58^{bc}$±7.87 | $60.60^{b}$±9.56 | $26.81^{b}$±4.42 | $28.37^{b}$±5.25 | $40.64^{c}$±2.02 | 52.68b±4.71 | $29.78^{b}$±0.88 | $15.52^{bc}$±0.93 | $37.95^{c}$±1.05 | $43.75^{c}$±3.58 |
| 1% | $29.42^{ab}$±3.33 | $48.56^{b}$±8.25 | $31.52^{b}$±1,28 | $56.11^{b}$±4.95 | $12.16^{b}$±1.47 | $29.00^{a}$±0.13 | $24.73^{ab}$±2.92 | $23.92^{a}$±4.28 | $10.37^{a}$±0.84 | $19.9^{ab}$±1.77 | $27.16^{b}$±1.66 | $22.14^{a}$±1.49 | $19.62^{b}$±1.14 | $9.10^{b}$±0.76 | $31.66^{b}$±1.12 | $33.80^{b}$±0.70 |
| control | $9.98^{a}$±1.42 | $4.30^{a}$±.29 | $2.42^{a}$±0.02 | $1.93^{a}$±0.08 | $8.27^{a}$±0.61 | $7.09^{a}$±1.08 | $2.57^{a}$±0.42 | $7.76^{a}$±0.60 | $5.12^{a}$±1.46 | $7.66^{a}$±0.72 | $7.53^{a}$±0.68 | $21.60^{a}$±0.93 | $2.41^{a}$±0.01 | $1.36^{a}$±0.76 | $3.39^{a}$±0.01 | $1.37^{a}$±0.01 |

Values are means of triplicate ± standard error. Column means followed by the same superscript letters are not significantly different at $P<0.05$.

## References


1.Adzu, B. Abubakar, M.S. Izebe, K.S. Akumka, D.D. and Gamaniel, K.S. (2005) Effect of *Annona senegalensis* rootbark extracts on *Najanigricotlis nigricotlis* venom in rats, *Journal of Ethnopharmacology,* 96: 507–513

2.Ahmad R., Jilani G., Arshad M., Zahir Z.A., Khalid A. (2007). Bioconversion of organic wastes for their recycling in agriculture: An overview of perspectives and prospects, *Annals of Microbiology,* 57 (4): 471-479.

3.Ajaiyeoba, E. Falade, M. Ogbole, O. Okpako, L. and Akinboye, D. (2006). In vivo antimalarial and cytotoxic properties of Annonasenegalensis extract. *African Journal of Traditional, Complementary and Alternative medicines,* 3 (1) 137 – 141

4.Audu, J. (1989). Medicinal herbs and their uses in Bauchi State, *The Nigerian Field*, 54: 157- 168.

5.Burkill, H. M. (1985). "The Useful Plants of West Africa," Royal Botanical Gardens, Kew, pp. 103–105.

6.Casimiro, G. S. Mansur, E. Pacheco, G. Garcia, R. Leal, I. C. R. and Simas, N. K. 2017. Allelopathic Activity of Extracts from Different Brazilian Peanut (Arachis hypogaea L.) Cultivars on Lettuce (Lactuca sativa) and Weed Plants, *The Scientific World Journal* ,17:1-7

7.Dalziel, J. M. (1937). The useful plants of West Tropical Africa.Crown overseas agents for the colonies, London, 2–3.

8.Durodola, J. I. (1975). Viability and transplanability of developed tumour cells treated in vitro with antitumour agent C/M2 isolated from herbal cancer remedy of *Annonasenegalensis*, *Planta Medica*, 28: 359.

9.Ekpenda, T. O. E. Obande, O. D. Anyogo P. O. and Attah, A. D. (1998). Nigerian ethnomedicine and medicinal plant flora – the Benue experience part 1, *Journal of Pharmaceutical Research and Development*, 3: 37–46.

10.Ezugwu, C.O and Odoh U.E (2003). Anticonvulsant activity of the root extract of *Annona senegalensis*, *Journal of Tropical Medical Plants,* 4(1): 51-55.

11.Fatope, M. O. Ibrahim, H. and Takeda, Y. (1993) Screening of higher plants reputed as pesticides using the brine shrimp lethality assay, *International Journal of Pharmacognosy,* 31: 250–254.

12.Ghulam, J., Shaukat, M., Arshad, N. C., Imran, H., Muhammad, A .Allelochemicals: sources, toxicity and microbial transformation in soil, *Annals of Microbiology*, 58 (3) 351-357 (2008)

13.Haramoto E.R. (2004). The effects of brassica cover crops on weed dynamics. M.Sc. Thesis. Department of Plant, Soil, and Environmental Sciences, the University of Maine. Pp. 57

14.Hong N.H., Xuan T.D., Tsuzuki E., Terao H., Matsuo M., Khanh T.D. (2004). Weed control of four higher plant species in paddy rice fields in Southeast Asia, *Journal of Agronomy and Crop Science*, 190: 59-64.

15.Igwe, A.C and Onabanjo, A.O. (1989). Chemotherapeutic effects of *Annona senegalensis* in Trypanosomabruceibrucei, *Annals of Tropical Medicine and Parasitology*, 83 (5) 527–534

16.Jacobson, M. Redfern, R. E. and Mills, G. D. (1975). Naturally occurring insect growth regulators Screening of insect and plant extracts as juvenile hormone mimics*, Lloydia,* 38: 455–472.

17.Jilani G., Akram A., Ali R.M., Hafeez F.Y., Shamsi I.H., Chaudhry A.N., Chaudhry A.G. (2007).Enhancing crop growth, nutrients availability, economics and beneficial rhizospheremicroflora through organic and bio fertilizers, *Annals of Microbiology,* 57 (2): 177-183.

18.Jolad , S. D. Hoffmann, K. H. Schram, K. H. Cole, J. R. Bates, R. B. and Tempesta, M.S. (1984). A new diterpene from *Cupressus goveniana var. abramasiana*: 5 beta-hydroxy-6-oxasugiol (Cupresol), *Journal of Natural Product,* 47: 983-987

19.Kudi, A. C. and Myint, S. H. (1999). Antiviral activity of some Nigerian medicinal plant extracts, *Journal of Ethnopharmacology,* 68: 289–294.

20.Langason, R.B.F. Akunyili, D.N. and Akubue, P.I. (1994). A Preliminary study of the gastrointestinal effects of some Nigerian medicinal plants, *Fitoterapia*, 65: 235-240.

21.Magassouba, F.B. Diallo, A. Kouyat´e, M. Mara, F. Mara, O. Bangoura, O. Camara, A,.Traor´e, S. Diallo, A. Zaoro, M. Lamah, K. Diallo, S. Camara, G. Traor´e, S. K´eita, A. Camara, M.K. Barry, R. K´eita, S. Oular´ea, K. Barry, M.S. Donzo, M. Camara, K. Tot´e, K. Berghe, V.D. Tott´e, J. Pieters, L. Vlietinck, A. J. and Bald´e, A.M. (2007). Ethnobotanical survey and antibacterial activity of some plants used in Guinean traditional medicine, *Journal of Ethnopharmacology,* 114: 44-53.

22.More, G. Tshikalange, T.E, Lall, N. Botha, F. and Meyer, J.J.M. (2008).Antimicrobial activity of medicinal plants against oral microorganisms, *Journal of Ethnopharmacology,*119: 473-477

23.Muanze, D.N. Kim, B.W. Euler, K.L. and Williams, L. (1994).Antibacterial and antifungal activities of nine medicinal plants from Zaire, *International Journal of Pharmacognosy*, 32: 337-345.

24.Ogbadoyi, E.O. Abdulganiy, A. O. Adama, T.Z. and Okogun, J. I. (2007). In vivo trypanocidal activity of *Annona senegalensis* Pers. leaf extract against *Trypanosoma bruceibrucei*, *Journal of Ethnopharmacology,* 112: 85-89.

25.Okoli, C. O. Onyeto, C. A. Akpa, B. P. Ezike, A. C. Akah, P. A. and Okoye, T. C. (2010) Neuropharmacological evaluation of *Annona senegalensis* leaves, *African Journal of Biotechnology,* 9 (49) 8435-8444,

26.Sahpaz, S. González, M.C Hocquemiller, R. Zafra-Polo, M.C. and Cortes, D. (1996).Annosenegalin and annogalene: two cytotoxic mono-

tetrahydrofuranacetogenins from *Annona senegalensis* and *Annona cherimolia*, *Phytochemistry,* 42 (1) 103-7
27.Sahpaz, S. Laurens, A. Hocquemiller, R. Cavé A. and Cortés, D. (1994).Senegalène, une nouvelle acétogénineoléfinique mono-tetrahydrofuranique des grains *d'Annonasenegalensis*, *Canadian Journal of Chemistry* 72: 1533-1536
28.Sofowora, E. A. and Adewunmi, C. O. (1980) Preliminary screening of some plant extracts for molluscidal activity, *Planta Medica,* 39: 57–65.
29.Sowemimo, A.A. Fakoya. F.A. Awopetu, I. Omobuwajo, O.R and Adesanya, S.A. (2007).Toxicity and mutagenic activity of some selected Nigerian plants. *Journal of Ethnopharmacology,* 113: 427 432
30.Suleiman, M.M. Dzenda, T. and Sanni, C.A. (2008). Antidiarrhoeal activity of the methanol stem-bark extract of *Annonasenegalensis* Pers. (Annonaceae), *Journal of Ethnopharmacology,* 116 (1) 125–130.