Original article:

ISOLATION OF 3-(4-HYDROXYPHENYL) METHYLPROPENOATE AND BIOACTIVITY EVALUATION OF GOMPHRENA CELOSIOIDES EXTRACTS

O. O. Dosumua,*, P. A. Idowuc, P. A. Onocha, O. Ekundayob

a Department of Chemistry, University of Ilorin, Ilorin, Nigeria
b Department of Chemistry, University of Ibadan, Ibadan, Nigeria
c Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria

* Corresponding author e-mail: oltados@yahoo.com, +234 805 614 8587, +234 703 611 4475

ABSTRACT

The efficacy of Gomphrena celosioides extracts in traditional medicine in the treatment of infectious diseases was evaluated by biological assays. The bioactivities of the extracts of this plant were tested against organisms. The ethyl acetate and methanol extracts of the plant displayed inhibition activities on Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi. Methanol extract was active against Candida albicans, Aspergillus niger and Trichophyton species with diameter zones of inhibition between 14 and 20 mm. Fractionation of the methanol extract produced 3-(4-hydroxyphenyl) methylpropenoate with mild antimicrobial activity against the test microorganisms. The anthelmintic assay showed ethyl acetate and methanol extracts to be active against Fasciola gigantica, Taenia solium and Pheretima pasthuma. Ethyl acetate was the most toxic of the extracts causing paralysis of Taenia solium within 15 minutes and the death of Fasciola gigantica within 20 minutes of application. The brine shrimp assay gave an LC 50 of 52.15 and 77.98 µg/ml on hexane and methanol extracts respectively. The result of this work corroborated the folkloric use of Gomphrena celosioides in the treatment of infectious diseases.

Keywords: Phytochemical analysis, antimicrobial, cytotoxicity, anthelmintic, Gomphrena celosioides

INTRODUCTION

Multiple drug resistance is a common problem nowadays in the treatment of internal parasites and infectious diseases due to abuse and indiscriminate use of drugs. A number of pathogens are already developing resistance to the available drugs (Davis, 1994; Service, 1995). The continued utilization of these drugs had resulted in hypersensitivity, immune suppression and allergic reactions (Ahmad et al., 1998). Pathogens resistance to available drugs is alarming (Bhavnani & Ballow, 2000), consequently, there is a need to search for new and effective therapeutic agents for the treatment of diseases caused by these organisms. Search for cure for these diseases from natural source is growing because of degradable potential of herbal drugs apart from efficacy. Ethno-botanical information of plant traditionally used for treating these diseases is of particular importance to drug discovery, so collaborative work with traditional healers is paramount in this direction.

Gomphrena celosioides belongs to the Amaranthacea family and over 120 species of the family are found in America, Austra-
lia and Indo-Malaysia, while 46 species occur in Brazil. Few species occur in the East and West of Africa (Vieira et al., 1994). Gomphrena species in different parts of the world are used for various folkloric medicinal purposes. In Brazil, some species are employed in the treatment of bronchial infections, diarrhea, and malaria fever, while others had found application as analgesic, tonic/carinminative and diuretics (Gessler et al., 1994; Vieira et al., 1994). Gomphrena celosioides is used in ethnomedical practice in Nigeria for treatment of various skin diseases, worms' infections and infectious diseases. In South America the plant is utilized as an abortifacient (Burkill, 1984). A decoction of the whole plant and a related species Gomphrena globosa is applied to gangrenous wound. G. martiana and G. boliviana are employed as antimicrobial agents by the natives (Arenac & Azorearo, 1977). Phytochemical investigation of G. martiana and G. boliviana led to the isolation of flavanones with antimicrobial properties (Pomilio et al., 1992).

Earlier research work by Botha and Gerritsma-Van der Vijver (1986) on Gomphrena celosioides extracts revealed the presence of saponins, steroids, amino acids, non-reducing sugars, phenols and flavonoids. Other workers had also isolated the above mentioned compounds and related compounds during the pharmacological studies of the plant (Vieira et al., 1994; Banerji et al., 1971; De Moura et al., 2004).

This present study isolated a bioactive compound from the methanol extract and carried out the biological studies, antimicrobial, anthelmintic and brine shrimp toxicity assays of the Gomphrena celosioides extracts.

**MATERIALS AND METHODS**

**General methods**

Infrared spectra were recorded on Bruker FT vector 22 spectrophotometer in KBr discs, in cm\(^{-1}\). EIMS (ionization voltage 70 ev) was measured on a Varian MAT 311/A mass spectrometer and HR EIMS were taken by MS JEOL-MS route (JMS-600H). \(^1\)H and \(^13\)C-NMR spectra were run on Bruker AMX400 and AMX500 MHz NMR spectrometers at 200(\(^1\)H) and 75(\(^13\)C). The chemical shifts are given in ppm (\(\delta\)) relative to TMS as internal standard and coupling constant, \(J\) in Hz.

In the biological assay, the inoculum size of the microorganisms was adjusted to 2.6 \(\times\) \(10^7\) cfu/ml using Unicam gamma 1000 spectrophotometer at 540 nm for the molten nutrient agar.

**Plant materials**

Fresh Gomphrena celosioides were collected in November 2005 from Abdulsalami Abubakar Graduate Hall, University of Ibadan, Nigeria. The identity was authenticated by Mr. Felix Nsong of Forest Research Institute of Nigeria (FRIN), Ibadan. A voucher specimen with herbarium number FHI 106429 was deposited in the FRIN herbarium.

**Extraction and isolation**

Air dried and ground G. celosioides (3.2 kg, whole plant) was exhaustively extracted with hexane, ethyl acetate and methanol successively. The extracts were concentrated under pressure to yield hexane (24.4 g), ethyl acetate (19.50 g) and methanol (93.80 g) extracts.

Crude methanol residue (10 g) was fractionated by vacuum liquid chromatography on silica gel (Merck). Partitioning was done in hexane followed by gradient mixtures of diethyl ether. Fractions were separated further on small column and 20 % ether in hexane afforded compound 1. Repeated crystallization in methanol afforded white crystal and structural elucidation resolved compound 1 as 3-(4-hydroxyphenyl) methylpropenoate.
δ6.85 and δ6.30 (2H each, dd, J = 9Hz), δ3.8 (3H, s, CH<sub>3</sub>), δ7.24 (1H, s, -OH)

- <sup>13</sup>C-NMR (75MHz, CDCl<sub>3</sub>): δ167.78 (C-1<sup>1</sup>), δ51.57 (-CH<sub>3</sub>O), δ157.57 (C-4), δ144.46 (C-3<sup>1</sup>), δ127.36 (C-2<sup>1</sup>), δ115.8 (C-6), δ115.3 (C-5), δ115.3 (C-3), δ115.6(C-2), δ129.93(C-1)

- HR EIMS m/z 178.1 (calculated for C<sub>10</sub>H<sub>10</sub>O<sub>3</sub>, 178.06)

- EIMS m/z (rel. int): 147.0 (100), 119.0 (40.0), 91.0 (13.2), 64.9 (32.6), 59.4 (49.2)

**Antimicrobial assay**

**Test organisms**

Laboratory strains of bacteria and fungi were obtained from Pharmaceutical Microbiology Department of Faculty of Pharmacy, University of Ibadan. Five bacteria, consisting of gram positive and gram negative were used in this study: *Staphylococcus aureus* NCTC6571, *Escherichia coli* NCTC9001, *Pseudomonas aeruginosa* NCTC6750, *Bacillus subtilis* (Lab-stock), *Salmonella typhi* ATCC14028 and three fungi, *Candida albicans* (NCTC 7534), *Aspergillus niger* and *Tricophyton species* (clinical isolates). Nutrient agar, sabouraud dextrose agar (SDA), tryptone soy agar (Oxoid Ltd, UK) were the media used in the assay. Dimethylsulphoxide (DMSO) (Merck) was used in dissolving the extracts and the same was used as negative control in the assay.

**Standard drugs**

Ampicillin (Beecham) 25 µg/ml, Tioconazole (Pfizer) 1 % w/v (0.5 mg/ml) and piperazine citrate (Pfizer) were used as reference drugs in the assay.

**Quantitative antimicrobial evaluation**

The agar cup diffusion method (Perez et al., 1990; Kavanagh 1972) was used. Two-fold dilutions of overnight broth cultures (0.1 ml) of organisms at inoculums size adjusted to 2.6 x 10<sup>7</sup> cfu/ml using Unicam gamma 1000 spectrophotometer at 540 nm were inoculated into cooled but molten nutrient agar (for fungal cultures, surface spread method was used for inoculation on SDA). The plates were allowed to solidify and wells were made using a sterile 7 mm diameter cork borer. The extracts (12.5 mg/ml) in DMSO were introduced into the wells with the aid of a dispenser. Controls and standards were set up containing the solvent (DMSO) and ampicillin (25 µg/ml) for bacteria and tioconazole (0.5 mg/ml of 1 % w/v) for fungi.

Diameters of zones of inhibition were determined after incubating plates at 37ºC for 24 h for bacteria and at 25ºC for 72 h for fungi. Antimicrobial studies were done in triplicates and diameters of zones of inhibition (mm) were expressed as the mean and standard deviations of the means. Zones of inhibition ≥ 10 mm were considered active (Zwadyk, 1972). Student ‘T’ tests was used to test for probability at P < 0.05.

**Anthelmintic assay**

*Fasciola gigantica* (liver fluke mean weight of 0.05 - 0.07 g) and *Taenia solium* (Tapeworm, 2.4 - 2.8 g) were obtained from freshly slaughtered cows at the Bodija abattoir, in Ibadan metropolis. *Pheretima pasthuma* (Earthworm, 0.06 – 0.6 g) were collected from the Awba dam and the water logged areas of Staff school, both within the campus of University of Ibadan. All the three types of worm were authenticated at the Parasitology Research Unit of Zoology Department in University of Ibadan.

Five worms of the same type were placed in 9 cm Petri dishes containing solutions of different concentration of the extracts made in DMSO (10, 20, 30, 40, 50, 70, 80, 100 mg/ml) respectively. This was done in duplicate for all worm types. Times of paralysis (P), in minutes were taken when no movement of any sort could be observed, except when the worms are shaken vigorously. Time of death of worms (D), in minutes were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50ºC). Mean time was calculated for P and D. Piperazine citrate
(10 mg/ml) was included as a reference drug while DMSO in distilled water served as control in accordance with modified Ajaiyeoba and Okogun methods (1996).

**Brine shrimp toxicity assay**

The brine shrimp lethality test was used to determine extracts and isolated compound toxicity (McLaughlin et al., 1993). 10 brine shrimp nauplii were introduced into vials containing 1000, 100 and 10 ppm concentration of the extracts and isolated compound. The effect of this extract on the brine shrimp nauplii were examined after 24 h. The ratio of dead nauplii was compared to the total number of nauplii. The result was analysed by Finney program and the LC50 calculated. Podophyllotoxin was used as a positive control in the bioassay.

**RESULTS AND DISCUSSIONS**

**Isolation**

Fractionation of the crude methanol residue afforded compound 1, a white crystal with melting point of 87°C. The IR spectrum of this compound showed absorption peaks at 3420, 2940, 2850, 1745, 1670, 1450 and 1375 cm⁻¹. The absorptions at 3420 and 1670 cm⁻¹ are characteristic of OH and C=O stretching vibrations respectively. Earlier workers have reported similar absorption frequencies for OH and C=O in vanillic acid (de Moura et al., 2004). The ¹H-NMR showed two pairs of characteristic doublets at 87.63 and 87.41 with large coupling constant (J = 15.95Hz) allotted to the olefinic α- and β-protons of the (E)-hydroxycinnamoyl moiety (Terahara et al., 2001). The ¹H-NMR exhibited two ortho and meta coupled protons with signals at δ6.85 and 6.25 (2H each, J= 9Hz) characteristic of a p-disubstituted benzene ring. The downfield shift of the former signal indicated the presence of an electron-withdrawing group on the benzene ring. A singlet at δ3.80 was due to methyl protons of the ester.

The ¹³C-NMR and DEPT analysis of the compound indicated that there are 10 carbon atoms in the molecule, three of which are quaternary (including a carbonyl), six are methine (CH) and one is methyl (CH₃). Signal at δ167.78 was due to carbonyl carbon, δ51.57 signal was that of the methyl carbon next to oxygen, while the carbinol carbon showed signal at δ157.57. The olefinic carbons had absorptions at δ144.46 and δ129.93.

The HR-EIMS gave molecular ion peak at 178.1 which translated to a compound with molecular formula of C₁₀H₁₀O₃. The EIMS measurement showed the base peak at m/z 147 (100 %) and this peak was resulted from the fragmentation of –OCH₃ ion from the molecular ion, while the peak at 119 (40.42 %) resulted from cleavage of –COOCH₃ ion from the parent molecule.

The IR, NMR and MS spectra data with a molecular mass of 178 coupled with the fragmentation pattern recorded elucidated compound 1 to be 3-(4-hydroxyphenyl) methylpropenoate with molecular formula of C₁₀H₁₀O₃. 3-(4-hydroxyphenyl) methylpropenoate is a derivative of vanillic acid which had earlier been isolated from other species of the family.

Table 1: NMR spectra data of 3-(4-hydroxyphenyl) methylpropenoate

| C/H | δC  | δH  |
|-----|-----|-----|
| 1   | 167.78 | -   |
| 2   | 129.93 | 7.41 |
| 3   | 144.46 | 7.63 |
| 1’  | 127.36 | -   |
| 4’  | 157.57 | -   |
| 2’/6’ | 115.85 | 6.85 |
| 3’/5’ | 115.40 | 6.25 |
| OCH₃ | 51.57 | 3.85 |

3-(4-hydroxyphenyl) methylpropenoate
Bioactivities

The presence of OH group in phenolic compounds had shown responsibility for inhibitory activities on microorganisms (Aziz et al., 1998; Friedman et al., 2003). This explained the antimicrobial activities recorded for this plant extracts and the isolated compound with an OH group. Gourma et al. (1989) explained that the OH group is very reactive and can easily form hydrogen bonds with active sites of enzymes. This was buttress with 0.4 mg/disc and 2.1 mg/ml of vanillic acid which completely inhibited the growth of Escherichia coli and its related strains (Aziz et al., 1998; Chamkha et al., 2002). Table 2 shows the antibacterial activity of crude ethyl acetate extract, methanol extract and 3-(4-hydroxyphenyl) methylpropenoate. The isolate, 3-(4-hydroxyphenyl) methylpropenoate, an ester of the vanillic acid inhibition activity is therefore not surprising, hence S. typhi, E. coli, P. aeruginosa, B. subtilis and S. aureus growth were curtail with diameter zone of inhibition of 9±0.2, 9±0.3, 10±0.5, 11±0.7 and 10±0.4 mm respectively at 25 µg/ml. The phenolic compounds inhibition activity has also been found to be proportional to the number of OH group present in the compound (Friedman et al., 2003). The crude extract which contain many compounds is likely to have more than one OH groups as obtained in the isolated compound and the high concentration of application of the extract are likely possibilities for the higher inhibitory activities recorded for the extract than the isolated compound.

Apart from higher number of OH groups that are present in the extracts, synergism may account for higher inhibitory activities of the extracts also and lack of it may be responsible for lower activity recorded for the isolated compound (Odebode et al., 2004). Ethyl acetate and methanol extracts displayed higher inhibitory activities on the microorganisms than the isolated compound (Table 2) for reasons stated above. The relatively lower inhibitory activities of the extracts recorded compared with the standard antibacterial drug, ampicillin in this study might probably be due to metabolism of the extracts by the bacteria which reduced their activities. A selective sensitivity was observed for S. aureus and S. typhi against P. aeruginosa, P. mirabilis and E. coli which were resistant to ethanol extract of G. celosioides and vanillic acid in a study conducted by De Moura et al., (2004). Metabolism of the extract by the bacteria was adduced to be responsible for this observation. Methanol extract (12.5 mg/ml) had pronounced effect on the activities of the fungi, C. albicans, A. niger and Trichophyton species, with higher activity on C. albican (20 ± 0.3 mm) (Table 3). The inhibition activities of the extracts on the fungi were comparable to that of reference drug, tioconazole.

Table 2: Antibacterial activity of crude ethyl acetate extract, methanol extract and 3-(4-hydroxyphenyl) methylpropenoate

| Microorganism | EtOAc extract b) | MeOH extract b) | Isolate c) | Ampicillin c) |
|---------------|-----------------|----------------|------------|--------------|
| S. typhi      | 12 ± 0.3        | 12 ± 0.3       | 9 ± 0.2    | 19 ± 0.2     |
| E. coli       | 12 ± 0.4        | 12 ± 0.3       | 9 ± 0.2    | 15 ± 0.5     |
| P. aeruginosa | 12 ± 0.7        | 12 ± 0.1       | 10 ± 0.5   | 18 ± 0.1     |
| B. subtilis   | 14 ± 0.4        | 13 ± 0.2       | 11 ± 0.7   | 22 ± 0.3     |
| S. aureus     | 13 ± 0.3        | 13 ± 0.2       | 10 ± 0.4   | 18 ± 0.4     |

a) Values are mean ± SD  
b) 12.5 mg/ml  
c) 25 µg/ml
Both ethyl acetate and methanol extracts (10 mg/ml) were very active in invoking paralysis and death of *F. gigantica*, *T. solium* and *P. pasthuma*. The methanol extract was more potent in causing paralysis of *P. pasthuma* than the ethyl acetate extract and reference drug, piperazine citrate. The time of death of *T. solium* and *P. pasthuma* by piperazine citrate, ethyl acetate and methanol extracts was similar but piperazine citrate used far less time to paralysis and caused death of *F. gigantica* than the extracts (Table 4).

Compounds with low LC50 (i.e. < 100 µg/ml) show indication for presence of cytotoxic and insecticidal compounds (Krishnaraju et al., 2005). Therefore, LC50 of 52.148 and 77.978 µg/ml recorded for hexane and methanol extracts respectively (Table 5) may suggest the presence of cytotoxic and/or insecticidal compounds in the extracts while 3-(4-hydroxyphenyl) methylpropenoate with LC50 of 110.654 indicates weak cytotoxicity. The low LC50 values of the extracts support the use of the aqueous extract of this plant in folk medicine for the treatment of gangrenous wounds.

### Table 3: Antifungal activity of crude methanol extract

| Microorganism       | EtOAc extract | Tioconazole |
|---------------------|---------------|-------------|
| *C. albican*        | 20 ± 0.3      | 28 ± 0.4    |
| *A. niger*          | 17 ± 0.5      | 22 ± 0.3    |
| *Trichophyton species* | 14 ± 0.1     | 19 ± 0.1    |

a) Values are mean ± SD  
b) 12.5 mg/ml  
c) 0.5 mg/ml of 1% w/v

### Table 4: Anthelmintic activity of ethyl acetate and methanol extracts (min)a)

| Helminths          | EtOAc extract | MeOH extract | Piperazine citrate |
|--------------------|---------------|--------------|--------------------|
| *F. gigantica*     | P 20 ± 0.8    | 40 ± 0.5     | 3 ± 0.2            |
|                    | D 35 ± 0.5    | 45 ± 0.1     | 5 ± 0.5            |
| *T. Solium*        | P 15 ± 0.4    | 37 ± 0.3     | 5 ± 0.5            |
|                    | D 40 ± 0.3    | 42 ± 0.3     | 40 ± 0.5           |
| *P. Pasthuma*      | P 40 ± 0.2    | 8 ± 0.9      | 20 ± 0.3           |
|                    | D > 60        | 60 ± 0.5     | 60 ± 0.5           |

a) mean time in minutes ± SD  
b) 10 mg/ml  
P = Mean time of helminth paralysis  
D = Mean time of helminth death

### Table 5: Brine shrimp assay of the extracts and 3-(4-hydroxyphenyl) methylpropenoate

| Extract/isolate | LC50, µg/ml, 24h |
|-----------------|------------------|
| Hexane          | 52.146           |
| Methanol        | 77.978           |
| 3-(4-hydroxyphenyl)methylpropenoate | 110.654 |
CONCLUSION

The activities shown by the extracts of the plant corroborate the use of the plant by the traditional healer for antimicrobial and cytotoxic purposes as in the treatment of wounds, skin diseases, bronchial infections, diarrhea and other microbes’ related diseases, and the good anthelmintic activity displayed by the plant extracts suggests the plant as a possible source of potent anthelmintic drug isolation of 3-(4-hydroxyphenyl) methylpropenoate is been reported for the first time in *G. celosioides* to the best of our knowledge.

ACKNOWLEDGEMENTS

The authors are grateful to H.E.J, Research Institute of Chemistry, Pakistan for the spectroscopic analyses. Mr Felix Usong (late), FRIN plant identification is appreciated. Efforts of Prof. T. Olugbade of CSL, Obefemi Awolowo University, Ile-Ife is gracefully acknowledged for spectra interpretation. OOD is grateful to University of Ilorin, Ilorin for the Staff Development Award.

REFERENCES

Ahmad I, Mehmood Z, Mohammed F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol 1998;62:183-93.

Ajaiyeoba EO, Okogun JI. Anthelmintic activity of a root extract of *Ritchica capsaroides* Var. Longipedicellata. Phytherapy Res 1996;10:436-7.

Arenac P, Azorearo RM. Plants of common use in Paraguayan folk medicine for regulating fertility. Econ Bot 1977;31:298-301.

Aziz NH, Farag SE, Mousa LA, Abo-Zaid MA. Comparative antibacterial and antifungal effects of some phenolic compounds. Microbios 1998;93(374):43-54.

Banerji A, Chintalwar GJ, Joshi NK, Chadha MS. Isolation of ecdysterone from Indian plants. Phytochemistry 1971;10:2225-6.

Bhavnani SM, Ballow CH. New agents for gram-positive bacteria. Curr Opin Microbiol 2000;3:528-34.

Botha S, Gerritsma-Van der Vijver LM. Pharmacocohemical study of *Gomphrena celosioides* (Amaranthaceae). Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie 1986;5(1):40-45.

Burkill HM. The useful plants of West Tropical Africa, Vol 1 (pp 61-2); Vol 2 (pp 337-8). Kew: Royal Botanical Gardens, 1984.

Chamkha M, Record E, Garcia J, Asther M, Labat M. Isolation from Shea cake digester of a tannin-tolerant *Escherichia coli* strain decarboxylating *p*-hydroxybenzoic and vanillic acid. Curr Microbiol 2002;44:341-9.

Davis J. Inactivation of the antibiotics and the dissemination of resistance genes. Science 1994;264:375-82.

De Moura XRM, Pereira PS, Januario AH, Franca S, Dias DA. Antimicrobial screening and quantitative determination of benzoic acid derivatives of *Gomphrena celosioides* by TLC-densitometry. Chem Pharm Bull 2004;52:1342-4.

Friedman M, Henika, PR, Mandrell RE. Antibacterial activities of phenolic benzaldehydes and benzoic acids against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica*. J Food Prot 2003;66:1811-21.

Gessler MC, Nkunya MH, Mwasumbi LB, Heinrich M, Tanner M. Screening Tanzanian medicinal plants for antimalarial activity. Acta Tropica 1994;56:65-77.
Gourma HLB, Tantawi LB, El-Araki M, Benbye KJ, Eullerman LB. Effect of oleuropein tyrosol and caffeic acid on the growth on mould isolated from the olive. J Food Prot 1989;43:264-6.

Kavanagh F. Analytical microbiology, Vol II. New York: Academic Press, 1972.

Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay H, Subbaraju GV. Assessment of bioactivity of India medicinal plants using brine shrimp (Artemia salina) lethality assay. Int J Appl Sci Engin 2005;32:125-34.

McLaughlin JL, Chang CJ, Smith DL. Simple bench-top bioassays (brine shrimp and potato discs) for the discovery of plant anti-tumour compounds. In Kinghorn AD, Balandrin MF (eds): Human medicinal agents from plants (pp 112-37). Washington, DC: American Chemical Society, 1993 (ACS Symposium, No. 534).

Odebode AC, Madachi SJM, Joseph CC, Irungu BN. Antimicrobial activities of constituents from Isolona cauliflora Verdc and Cleistochlamys krikii Benth (Oliv) (Annonaceae). J Agric Sci 2004;49:109-16.

Perez C, Pauli M, Bazerque P. An antibiotic assay by agar well diffusion method. Acta Biol Med Exp 1990;15:113-5.

Pomilio AB, Buschi CA, Tomes CN, Viale AA. Antimicrobial constituents of Gomphrena martiana and Gomphrena bolivi-ana. J Ethnopharmacol 1992;36:155-61.

Service RF. Antibiotics that resist resistance. Science 1995;270:724-7.

Terahara N, Takeda Y, Nesumi A, Honda T. Anthocyanins from red flower tea (Beni-bana-cha), Camellia sinensis. Phytochemistry 2001;56:359-61.

Vieira CCJ, Mercier H, Chu EP, Figueiredo-Ribeiro RCL. Gomphrena species (globe amaranth): In vitro culture and production of secondary metabolites. In Bajaj YPS (ed): Biotechnology in agriculture and forestry (pp 257-70). Berlin: Springer-Verlag, 1994.

Zwadyk P. Enterobactericeae: Salmonella and Shigella, intestinal pathogens. In: Joklik WK, Willett HP, Mos DB (eds): Zinsser microbiology. 20th ed. (pp 544-6). Stuttgart: Thieme, 1972.