

Comparative in-vitro anticancer and brine shrimp cytotoxic activities of *Mezoneuron benthamianum* Baill.



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Background: Cancer is one of the leading causes of global mortality and recently, it has been established that there is a link between periodontal disease and various types of cancer. In Nigeria, chewing sticks are used especially in the rural areas to maintain oral hygiene and to prevent periodontal disease. *Mezoneuron benthamianum* is a plant that is used locally as a chewing stick in the southwest of Nigeria, but there has been no report on its anticancer properties.

Aim: This study is aimed at determining the anticancer activity using sulforhodamine B (SRB) assay and brine shrimp cytotoxic activity of the extracts of *M. benthamianum*.

Setting: The roots of *M. benthamianum* were obtained from Ibadan, Oyo State, Nigeria, and were identified and authenticated at the University of Ibadan Herbarium.

Methods: The plant sample was subsequently dried, pulverised and extracted with hexane, dichloromethane, ethyl acetate and methanol to give the different extracts which were tested against four cell lines (Lung A549, Lung NCI-H322, Breast T47D and Prostate PC-3) using the SRB assay and were also evaluated against brine shrimp *nauplii*.

Results: The results of the study showed that the different extracts of *M. benthamianum* had selective and consistent cytotoxic activity against the Lung (A549), Lung (NCI-H322) and Breast (T47D) cell lines, having a percentage growth inhibition ranging from 36% to 63%. The hexane and dichloromethane extracts also gave LC_{50} values of 99.96 and 29.29 against brine shrimp cytotoxic activity.

Conclusion: These results justify the use of *M. benthamianum* in folkloric medicine.

Keywords: brine shrimp; cytotoxicity; *Mezoneuron benthamianum*; chewing sticks.

Introduction

Oral diseases, including oral and pharyngeal cancers, tooth loss, dental caries and periodontal disease, are a global health concern. Periodontal disease is a chronic inflammatory disease resulting in the destruction of tissues and structures surrounding the teeth. It has been established that there is a link between periodontal disease and various types of cancer (Grant & Boucher 2011; Hujoel et al. 2003).

The sulforhodamine B (SRB) assay is an assay that is used to access the cytotoxicity property of plant extracts through the determination of cell density, which is based on the measurement of cellular protein content. The assay relies on the ability of the SRB to bind with basic amino acid residues of trichloroacetic acid (TCA)-fixed protein contents of the cells (Vichai & Kirtikara 2006). The SRB is simple, sensitive, rapid and reproducible and has a stable end point that does not require a time-sensitive measurement (Houghton et al. 2007).

The brine shrimp lethality bioassay is a rapid and comprehensive bioassay for bioactive compounds of natural and synthetic origin. It is an important tool for the preliminary cytotoxicity assay of plant extract, based on its ability to kill the brine shrimp *nauplii*. This method enables plant extracts to be accessed for their bioactivity. The method is convenient and can be used to monitor the screening and fractionation of new bioactive natural products from plants. Brine shrimp toxicity is

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closely correlated with human nasopharyngeal carcinoma cytotoxicity. Accordingly, it is possible to detect and then monitor the fractionation of cytotoxic extracts using the brine shrimp lethality bioassay (Alkofahi et al. 1988; McLaughlin, Rogers & Anderson 1998).

Mezoneuron benthamianum Baill. is a shrub in the secondary jungle and savanna forest ranging from Senegal to Nigeria. It is a climbing or a straggling shrub. The leaf is administered as a mild laxative in Senegal in treating enteralgia. The root is reportedly used in the Ibadan area of Nigeria as a chewing stick with the claim of effectiveness against dental caries (Burkill 1985). Several studies on *M. benthamianum* have focused on its antibacterial, antifungal, anti-inflammatory, antioxidant and antidiarrhoeal activities (Fayemi & Osho 2012; Mbagwu & Adeyemi 2008; Zambé et al. 2008), but there has been no reports of anticancer activities on cancer cell lines or cytotoxic activities on brine shrimp nauplii. Therefore, this study is aimed at determining the cytotoxic activities of the crude extracts of *M. benthamianum* using SRB and brine shrimp lethality assay.

Materials and methods

General experimental procedure

The solvents used for extraction were laboratory grade chemicals and were purified prior to use. Reagents used for the SRB cytotoxic assay were: Roswell Park Memorial Institute medium (RPMI-1640), foetal calf serum, trypsin, phosphate buffer saline (PBS), trypan blue, penicillin, streptomycin, Dimethyl sulfoxide (DMSO), sulforhodamine, paclitaxel (taxol) and 5-fluorouracil, which were obtained from Sigma Chemical Corp., United States. Materials used for the brine shrimp cytotoxicity assay were: brine shrimp (*Artemia salina*), eggs and salt, which were purchased from Essex Marine Aquatics, United Kingdom.

Plant preparation

The roots of *M. benthamianum*. Baill. were obtained in Ibadan, identified and authenticated at the Department of Botany, University of Ibadan, Nigeria, where a voucher specimen has been deposited in the herbarium (No: UIH-22401).

Plant extraction

The dried, pulverised sample of *M. benthamianum* (2.74 kg) was extracted twice using the cold extraction method by maceration with 10 L of methanol for 3 days each. The extracts were concentrated using a rotary evaporator under reduced pressure to give a dark brownish solid (200 g). This was then dissolved in aqueous methanol and was further partitioned with hexane, dichloromethane and ethyl acetate.

In-vitro anticancer assay

The crude extracts from *M. benthamianum* were screened against different cell lines such as Lung (A549), Prostate

(PC-3), Lung (NCI-H322) and Breast (T47D) using the SRB assay (Jain et al. 2014; Kaur et al. 2014; Monks et al. 1991).

Briefly, the human cancer cell lines were grown in tissue culture flasks in a complete growth medium (RPMI-1640 medium with 2 mM glutamine, pH 7.4, supplemented with 10% foetal calf serum, 100 µg/mL streptomycin and 100 units/mL penicillin) in a carbon dioxide incubator (37 °C, 5% CO₂, 90% relative humidity [RH]). The cells at the subconfluent stage were harvested from the flask by treatment with trypsin (0.05% in PBS [pH 7.4] containing 0.02% Ethylene diamine tetraacetic acid (EDTA)). Cells with a viability of more than 98%, as determined by trypan blue exclusion, were used for the determination of cytotoxicity. The cell suspension of 1 × 10⁵ cells/mL was prepared in a complete growth medium. Stock solutions (20 mM/mL) of compounds were prepared in DMSO. The stock solutions were serially diluted with the complete growth medium to obtain working test solutions of required concentrations.

In-vitro cytotoxicity against human cancer cell lines was determined using 96-well tissue culture plates. The 100 µL of cell suspension was added to each well of the 96-well tissue culture plate. The cells were allowed to grow in a carbon dioxide incubator (37 °C, 5% CO₂, 90% RH) for 24 hours. Test materials in a complete growth medium (100 µL) were added after 24 h of incubation to the wells containing cell suspension. The plates were further incubated for 48 h in a carbon dioxide incubator. The cell growth was stopped by gently layering TCA (50%, 50 µL) on top of the medium in all of the wells. The plates were incubated at 4 °C for 1 h to fix the cells attached to the bottom of the wells. The liquid of all the wells was gently pipetted out and discarded. The plates were washed five times with distilled water to remove TCA, low molecular weight metabolites, serum proteins and so forth and were air-dried. The plates were stained with SRB dye (0.4% in 1% acetic acid, 100 µL) for 30 min. The plates were washed five times with 1% acetic acid and then air-dried (Skehan et al., 1990). The adsorbed dye was dissolved in a Tris-HCl buffer (100 mL, 0.01 M, pH 10.4) and plates were gently stirred for 10 min on a mechanical stirrer. The optical density (OD) was recorded on an ELISA reader at 540 nm. The cell growth was determined by subtracting the mean OD value of the respective blank from the mean OD value of the experimental set. The percentage growth in the presence of test material was calculated considering the growth in the absence of any test material as 100%, and in turn the percentage growth inhibition in the presence of test material was calculated.

$$\% \text{ Growth inhibition} = 100 - \left[\frac{OD_{(\text{Test sample})} - OD_{(\text{Blank})}}{OD_{(\text{Control})} - OD_{(\text{Blank})}} \times 100 \right] \quad [\text{Eqn 1}]$$

Brine shrimp cytotoxic assay

Brine shrimp eggs, *Artemiasalina* sp. were hatched in a vessel containing distilled water to which the salt and eggs were added. The vessel was kept under an inflorescent bulb and facilitated with good aeration for 48 h at room temperature.

After hatching, nauplii released from the egg shells were collected at the bright side of the vessel (near the light source) by using a micropipette. The stock solution of 1000 parts per million (ppm) was prepared by weighing 20 mg of the sample into 2 mL of 1% DMSO in salt water. Serial dilutions of 100 ppm and 10 ppm were prepared by transferring 0.2 mL of the stock solution to 1.8 mL of 1% DMSO in salt water, in another test tube, to give 100 ppm, which was also further diluted to give 10 ppm. This was done in triplicate. After 2 days (when the shrimp larvae were ready), 4 mL of salt water was added to each test tube and 10 shrimps were introduced into the test tube, after which the volume was adjusted with salt water to 5 mL/test tube. The test tubes were placed, uncovered, under the lamp and the set-up was allowed to continue for 24 h, after which the number of survivors were counted and recorded. The percentage mortality was calculated as the ratio of the number of dead nauplii to the initial number of nauplii multiplied by 100. The results were analysed with the Finney computer program for probit analysis to determine the LC_{50} values at 95% confidence intervals or alternatively obtained by a plot of the percentage of shrimps killed against the logarithm of the sample concentration. The LC_{50} values were used to determine the lethality of the extracts, and values greater than 1000 ppm were considered inactive (McLaughlin et al. 1998).

Ethical consideration

Ethical clearance was not needed/required for the study.

Results and discussion

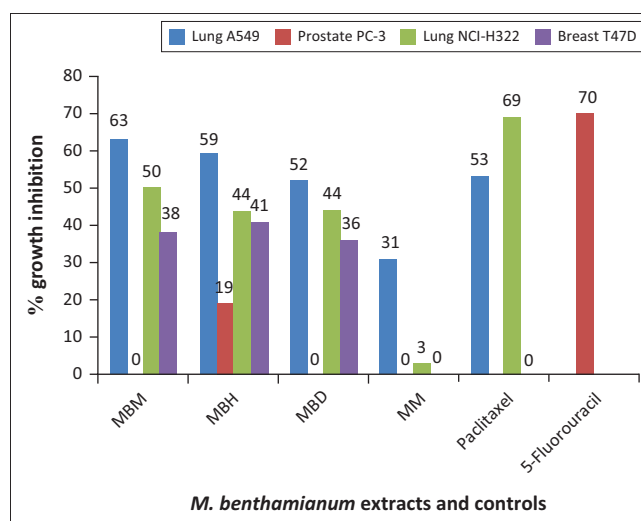
Plant extraction

The results of the extraction and partitioning of the methanol extract of *M. benthamianum* into hexane, dichloromethane, ethyl acetate and aqueous methanol are shown in Table 1 below.

Anticancer activities

The SRB assay relies on the uptake of the negatively charged pink aminoxanthine dye, SRB by basic amino acids in the cells. The greater the number of cells, the greater the amount of dye is taken up. After fixing, when the cells are lysed, the released dye will give a more intense colour and greater absorbance (Skehan et al. 1990). The SRB assay is sensitive, simple, reproducible and more rapid than the formazan-based assays and gives better linearity, a good signal-to-noise ratio and has a stable end point that does not require a time-sensitive measurement (Fricker & Buckley 1996).

The cell lines employed in this study were Lung NCI-322, Lung A549, Breast T47D and Prostate PC-3 cells. The results of the in-vitro cytotoxic activities (Figure 1) indicated that the extracts of *M. benthamianum* showed selectively consistent activity against Lung (A549 and NCI-322) and Breast (T47D) cancer cell lines, with crude methanol (MBM) having the highest percentage growth inhibition of 63%, 50% and 28%, whilst aqueous methanol extract (MBAM) had the least percentage growth inhibition of 31%, 3% and 0%, respectively. Dichloromethane (MBD) and hexane (MBH) extracts had an average percentage growth inhibition with a percentage growth inhibition of 59%, 44% and 41%, and 52%, 44% and 36%, respectively. They were all inactive against the prostate (PC-3) cancer cell line except MBH with a weak activity and a percentage growth inhibition of 19%. The results were also comparable to the positive control (Paclitaxel), which had a percentage growth inhibition against Lung (A549 and NCI-322) cell lines with a percentage growth inhibition of 53% and 69%, respectively. This result is comparable with the results from our previous study, which showed that the extracts of *M. benthamianum*, although less cytotoxic than that of *Fagara zanthoxylum* (percentage inhibition = 72%, 79%, 79% and 71% against Lung A549, Lung NCI-322, Breast T47D and Prostate PC-3, respectively), has comparative activity with *Nauclea latifolia* (% inhibition = 62%, 70%, 54% and 28% against Lung A549, Lung NCI-322, Breast T47D and Prostate PC-3, respectively).



MBM, *Mezoneuron benthamianum* unpartitioned methanol extract; MBH, *Mezoneuron benthamianum* hexane extract; MBD, *Mezoneuron benthamianum* dichloromethane extract; MM, *Mezoneuron benthamianum* methanol extract.

FIGURE 1: Percentage growth inhibition of the *Mezoneuron benthamianum* extracts against cancer cell lines.

Table 1: Percentage yield and physical appearance of *M. benthamianum* extracts.

S/No	Plant extract	Plant code	Physical appearance	Amount of plant sample (kg)	Amount of extract (g)	Yield (%)
1	Crude methanol extract	MBM	Dark brown solid	2.74	200.0	7.3
2	Hexane extract	MBH	Light yellow semi-solid	2.74	5.0	0.18
3	Dichloromethane extract	MBD	Brown solid	2.74	14.0	0.51
4	Ethyl acetate extract	MBE	Dark brown solid	2.74	70.0	2.55
5	Aqueous methanol extract	MBAM	Dark brown solid	2.74	100.0	3.65

MBM, crude methanol extract; MBH, hexane extract; MBD, dichloromethane extract; MBE, ethyl acetate extract; MBAM, aqueous methanol extract.

and more cytotoxic activity than *Butyrospermum paradoxum* and *Distemonanthus benthamianus* (Osamudiamen et al. 2017). The cytotoxic activity of *M. benthamianum* can be attributed to the presence of bioactive compounds, some of which include polyphenols such as resveratrol and piceatannol; flavonoids such as kaempferol and quercetin; and terpenoids such as taepeenin A and nortaepeenin A (Jansen et al. 2017; Osamudiamen et al. 2017, 2020). These compounds have been found to exhibit different degrees of anticancer activities. For instance, resveratrol has been found to exhibit anticancer activities, as it has been employed as an alternative drug to treat different cancers. Many reports have indicated that resveratrol provides a wide range of preventive and therapeutic options against different types of cancer. And it has been widely envisioned as a potentially useful candidate for anticancer therapy when combined with other chemotherapeutic drugs (Rauf et al. 2018). In addition, piceatannol has also been reported to provide a wide variety of preventive and therapeutic options against cancer (Jeong et al. 2015). It has also been found to possess greater biological activity compared to resveratrol (Potter et al. 2002). Also, kaempferol has been observed to augment the body's antioxidant defence against free radicals, which promote the development of cancer (Calderon-Montano et al. 2011). Finally, taepeenin A and nortaepeenin A, which were isolated from the hexane extract, have also been found to possess anticancer properties (Osamudiamen et al. 2017).

Brine shrimp cytotoxic activities

The result of the brine shrimp cytotoxic assay (Figure 2) shows that dichloromethane (MBD) and hexane (MBH) extracts had the greatest activity against the brine shrimp nauplii, with a lethal concentration (LC_{50}) of 29.29 mg/mL and 99.96 mg/mL, respectively. The crude methanol extract (MBM) had the least activity, with an LC_{50} of 374.02, whilst the aqueous methanol (MBAM) and ethyl acetate extracts (MBE) had an average activity, with an LC_{50} of 130.64 and

208.35, respectively. However, the results show that the extracts of *M. benthamianum* were active overall, as these all have LC_{50} values < 1000, which are considered significantly active (McLaughlin et al. 1998). These results can be correlated with our previous report of the cytotoxic activity of the isolated polyphenols from *M. benthamianum*, such as resveratrol and piceatannol, which were found to be potent cytotoxic agents (Osamudiamen et al. 2020).

Conclusion

This brine shrimp cytotoxic assay has a good correlation with the anticancer cytotoxic assay, as the non-polar extracts (hexane and dichloromethane extracts), which had the least LC_{50} values, also showed good cytotoxic activities against the Lung (A549 and NCI-322) and Breast (T47D) cell lines. The in-vitro cytotoxic activities of the crude extracts show that they possess significant cytotoxic activities against lung and breast cancer cell lines which have been linked to periodontal disease. They also show significant cytotoxic activities against brine shrimp nauplii and justify the use of *M. benthamianum* in oral health care. A further study on the activity of extracts of *M. benthamianum* against cell lines from the oral cavity is recommended.

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Competing interests

The authors have declared that no competing interests exist.

Author's contributions

All authors contributed equally to this work.

Funding information

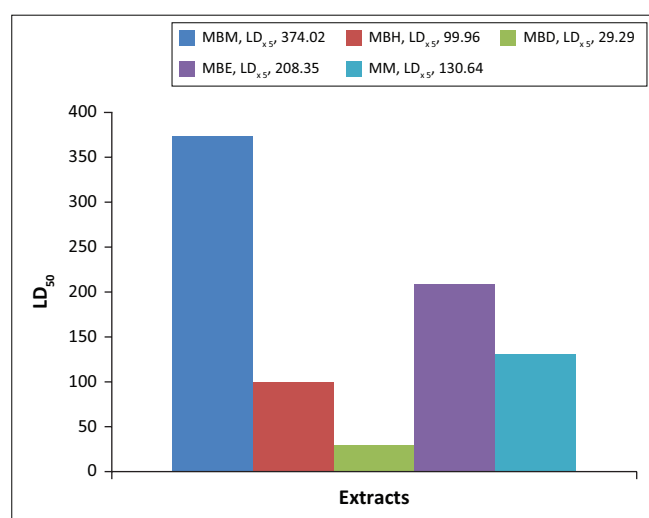
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Data availability statement

The data are available for sharing on request from the corresponding author.

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MBM, crude methanol extract; MBH, hexane extract; MBD, dichloromethane extract; MBE, ethyl acetate extract; MM, methanol residue.

FIGURE 2: Brine shrimp activity (LC_{50} values) of the extracts of *Mezoneuron benthamianum*.

References

- Alkofahi, A., Rupprecht, J.K., Smith, D.L., Chang, C.J. & McLaughlin, J.L., 1988, 'Goniothalamicin and annonacin: Bioactive acetogenins from *Goniothalamus gardneri*', *Phytochemistry* 49(5), 1317–1321.
- Burkill, H.M., 1985, *The useful plants of West tropical Africa*, 2nd edn., vol. 1, p. 91, Royal Botanic Gardens, Kew, Richmond.
- Calderon-Montano, J.M., Burgos-Morón, E., Pérez-Guerrero, C. & López-Lázaro, M., 2011, 'A review on the dietary flavonoid kaempferol', *Mini Reviews in Medicinal Chemistry* 11(4), 298–344. <https://doi.org/10.2174/138955711795305335>
- Fayemi Scott, O. & Osho, A., 2012, 'Comparison of antimicrobial effects of *Mezoneuron benthamianum*, *Heliotropium indicum* and *Flabellaria paniculata* on *Candida* species', *Journal of Microbiology Research* 2(1), 18–23. <https://doi.org/10.5923/j.microbiology.20120201.04>
- Fricker, S.P. & Buckley, R.G., 1996, 'Comparison of two colorimetric assays as cytotoxicity endpoints for an in vitro screen for antitumour agents', *Anticancer Research* 16(6B), 3755–3760.
- Grant, W.B. & Boucher, B.J., 2011, 'Low vitamin D status likely contributes to the link between periodontal disease and breast cancer', *Breast Cancer Research and Treatment* 128(3), 907–908. <https://doi.org/10.1007/s10549-011-1458-6>
- Houghton, P., Fang, R., Techatanawat, I., Steventon, G., Hylands, P.J. & Lee, C.C., 2007, 'The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity', *Methods* 42(4), 377–387. <https://doi.org/10.1016/j.ymeth.2007.01.003>
- Hujoel, P.P., Drangsholt, M., Spiekerman, C. & Weiss, N.S., 2003, 'An exploration of the periodontitis–cancer association', *Annals of Epidemiology* 13(5), 312–316. [https://doi.org/10.1016/S1047-2797\(02\)00425-8](https://doi.org/10.1016/S1047-2797(02)00425-8)
- Jain, U.K., Bhatia, R.K., Rao, A.R., Singh, R., Saxena, A.K. & Sehar, I., 2014, 'Design and development of halogenated chalcone derivatives as potential anticancer agents', *Tropical Journal of Pharmaceutical Research* 13(1), 73–80. <https://doi.org/10.4314/tjpr.v13i1.11>
- Jansen, O., Tchinda, A.T., Loua, J., Esters, V., Cieckiewicz, E., Ledoux, A. et al., 2017, 'Antiplasmodial activity of *Mezoneuron benthamianum* leaves and identification of its active constituents', *Journal of Ethnopharmacology* 203, 20–26. <https://doi.org/10.1016/j.jep.2017.03.021>
- Jeong, S.-O., Son, Y., Lee, J.H., Cheong, Y.-K., Park, S.H., Chung, H.-T. et al., 2015, 'Resveratrol analog piceatannol restores the palmitic acid-induced impairment of insulin signaling and production of endothelial nitric oxide via activation of anti-inflammatory and antioxidative heme oxygenase-1 in human endothelial cells', *Molecular Medicine Reports* 12(1), 937–944. <https://doi.org/10.3892/mmr.2015.3553>
- Kaur, R., Chattopadhyay, S.K., Chatterjee, A., Prakash, O., Khan, F., Suri, N. et al., 2014, 'Synthesis and in vitro anticancer activity of brevifolol derivatives substantiated by in silico approach', *Medicinal Chemistry Research* 23(9), 4138–4148. <https://doi.org/10.1007/s00044-014-0980-6>
- Mbagwu, H.O.C. & Adeyemi, O.O., 2008, 'Anti-diarrhoeal activity of the aqueous extract of *Mezoneuron benthamianum* Baill (Caesalpiniaceae)', *Journal of Ethnopharmacology* 116(1), 16–20. <https://doi.org/10.1016/j.jep.2007.10.037>
- McLaughlin, J.L., Rogers, L.L. & Anderson, J.E., 1998, 'The use of biological assays to evaluate botanicals', *Drug Information Journal* 32(2), 513–524. <https://doi.org/10.1177/009286159803200223>
- Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paull, K., David, V. et al., 1991, 'Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines', *Journal of the National Cancer Institute* 83(11), 757–766. <https://doi.org/10.1093/jnci/83.11.757>
- Osamudiamen, P.M., Aiyelaagbe, O.O., Koul, S., Sangwan, P.L., Vaid, S. & Saxena, A.K., 2017, 'Isolation, characterization, and in-vitro anti-cancer activity of bioactive cassane diterpenoids from the roots of *Mezoneuron benthamianum* (Baill.)', *Journal of Biologically Active Products from Nature* 7(3), 157–165. <https://doi.org/10.1080/22311866.2017.1335232>
- Osamudiamen, P.M., Oluremi, B.B., Oderinlo, O.O. & Aiyelaagbe, O.O., 2020, 'Trans-resveratrol, piceatannol and gallic acid: Potent polyphenols isolated from *Mezoneuron benthamianum* effective as anticaries, antioxidant and cytotoxic agents', *Scientific African* 7, e00244. <https://doi.org/10.1016/j.sciaf.2019.e00244>
- Potter, G.A., Patterson, L.H., Wanogho, E., Perry, P.J., Butler, P.C., Ijaz, T. et al., 2002, 'The cancer preventative agent resveratrol is converted to the anticancer agent piceatannol by the cytochrome P450 enzyme CYP1B1', *British Journal of Cancer* 86(5), 774–778. <https://doi.org/10.1038/sj.bjc.6600197>
- Rauf, A., Imran, M., Butt, M.S., Nadeem, M., Peters, D.G. & Mubarak, M.S., 2018, 'Resveratrol as an anti-cancer agent: A review', *Critical Reviews in Food Science and Nutrition* 58(9), 1428–1447. <https://doi.org/10.1080/10408398.2016.1263597>
- Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D. et al., 1990, 'New colorimetric cytotoxicity assay for anticancer-drug screening', *JNCI: Journal of the National Cancer Institute* 82(13), 1107–1112. <https://doi.org/10.1093/jnci/82.13.1107>
- Vichai, V. & Kirtikara, K., 2006, 'Sulforhodamine B colorimetric assay for cytotoxicity screening', *Nature Protocols* 1(3), 1112–1116. <https://doi.org/10.1038/nprot.2006.179>
- Zamblé, A., Martin-Nizard, F., Sahpaz, S., Hennebelle, T., Staels, B., Bordet, R. et al., 2008, 'Vasoactivity, antioxidant and aphrodisiac properties of *Caesalpinia benthamiana* roots', *Journal of Ethnopharmacology* 116(1), 112–119. <https://doi.org/10.1016/j.jep.2007.11.016>