

PHYTOCHEMICAL SCREENING AND *IN-VITRO* CYTOTOXIC ACTIVITIES OF METHANOLIC EXTRACTS OF *MEZONEURON BENTHAMIANUM* BAILL. AGAINST CANCER CELLS LINES

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Abstract: Despite the significant gains made in cancer therapy, cancer remains a major cause of global deaths due to rapid drug resistance. Therefore, urgent concerted efforts towards the discovery and development of newer and effective anticancer agents cannot be overemphasized. This study investigated *in vitro* cytotoxicity potential of methanol extracts of the root, stem, and leaves of *Mezoneuron benthamianum*. Leaf, stem and root samples were collected, authenticated, dried, separately pulverized and extracted in methanol. The methanol extracts were analysed for the presence of phytochemicals and cytotoxic potential evaluated by tetrazolium 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay on selected human cancer cells lines, HeLa (cervical cancer) and HEp-2 (epidermal carcinoma of the larynx), using mammalian Vero cells as a negative control. Data generated was subjected to descriptive statistics. The flavonoids in the plant was between 40 - 67.2% and was significantly higher ($p < 0.05$) compared to alkaloids and saponins. At concentrations of $\geq 50 \mu\text{g/mL}$, the extracts exhibited 100% cytotoxicity on the cancer cells. The methanol root and leaf extracts with CC_{50} of 15.64 and 11.38 $\mu\text{g/mL}$ were more cytotoxic on HeLa and HEp-2, respectively. In comparison to the stem and root extracts, the methanol leaf extract was selectively more toxic to cancer cell lines than Vero cells ($\text{CC}_{50} = 28.89 \mu\text{g/mL}$). Preliminary investigation reveals that *Mezoneuron benthamianum* contain bioactive compounds that possess promising anticancer potential that could be exploited.

Keywords: cancer cells, chemotherapy, *Mezoneuron benthamianum*, MTT

1. Introduction

Cancer is one of the leading causes of death in the world today. It is a chronic disease that occurs when cell growth gets out of control and divide uncontrollably to form lumps or

masses of tissue called tumors. The tumor causes damage to healthy tissues *in vitro* that requires further investigation and exploitation (Avni *et al.*, 2008). Several plant derived

compounds such as vinca alkaloids, podophyllotoxin derivatives, taxanes, camptothecin, combretastatin, among others are used in various regimens as chemotherapy of various cancers (Anna and Krzysztof, 2018).

Although great advancements have been made in the development of anticancer drugs and the treatment, however, severe undesirable adverse effects associated with many of the chemotherapeutic regimens and resistance to anticancer drugs are common phenomena. The discovery and development of plant-derived effective and broad spectrum chemotherapeutics with less adverse effects will be valuable options in the cancer chemotherapy (Avni *et al.*, 2008), and (Sudhakar, 2009). Many plants have shown promising anti-cancer properties.

Mezoneuron benthamianum is a climbing shrub that belongs to the family leguminosae (Fabaceae). It contains hydroxystilbenes, piceatannol and *trans*-resveratrol, which have been reported to possess chemopreventive properties (Osamudiamen *et al.*, 2020). This plant has been used in folk medicines to treat many diseases including general malaise, urethral discharge, inflammation, dropsy, swellings, oedema, cataract, wounds, skin infections, piles and ulcers (Burkill, 1985). In addition, plants belonging to the Fabaceae family have been reported to possess phytochemicals with anticancer activities and the phytochemical components of these plants inhibit carcinogenesis at various stages (Sharma *et al.* 2017). Previous reports also showed that the hexane extract of the root of *M. benthamianum* possesses phytochemicals that have *in vitro* cytotoxic activities against lung (NCI-H322), breast (T47D), prostate (PC-3) and lung (A549) cancer cell lines (Osamudiamen *et al.*, 2017). However, cytotoxic activities of the leaves and stem bark of *M. Benthamianum* have not been investigated. This study was therefore designed

to investigate the cytotoxic potential of the methanol extracts of the root, stem, and leaves of *Mezoneuron benthamianum* against HeLa (cervical cancer) and HEP-2 (epidermal carcinoma of the larynx).

2. Materials and methods

Collection of plant parts

The leaves, stem and roots of *Mezoneuron benthamianum* were collected from Afin-Iyanu area, Ologun-Eru, Ibadan. A mature part of the freshly collected plant with leaves, flowers and stem was authenticated at the herbarium unit of Department of Botany, University of Ibadan. The fresh leaves were spread in open air under a shade and away from direct sunlight, while the roots and stems were size reduced and air dried.

Extraction, qualitative and quantitative determination of phytochemicals in *M. benthamianum*

Dried leaves, roots, and stem of *M. benthamianum* were pulverized and extracted by cold maceration. The plant parts were weighed separately into separate glass containers and sufficient methanol was added and left to stand with stirring at intervals for 72 hours. The methanol extracts were filtered and concentrated under reduced pressure using rotary evaporator at 40°C. The extracts were stored in labeled bottles at room temperature until further use. The presence or otherwise of phytochemical compounds in each extract was qualitatively determined using standard methods described by Oluremi *et al.* (2018).

Preparation and Maintenance of the Cell lines

Epidermoid carcinoma of the larynx (HEP-2), cervical cancer (HeLa) and African monkey kidney cells (Vero) used in this study were obtained from the WHO Polio Laboratory,

Department of Virology, University of Ibadan, Ibadan. Each cell line was cultured in separate T/45 tissue culture flasks using 10% (growth) medium and incubated in a 5% humidified CO₂ incubator at 37°C until they were 80% confluent.

Determination of the cytotoxicity of *Mezoneuron benthamianum* extracts by MTT colorimetric assay

Sterile 96-well tissue culture microtiter plates were seeded with each cell lines. The plates were incubated in a 5% humidified CO₂ incubator at 37°C for 24 hours to attain 80-100% cell confluence. Spent media in the 96-wells were removed by aspiration and replenished with 100 µL of the different dilutions (0.001-100 µg/mL) of the methanol extracts in maintenance medium and the plates were further incubated. At the end, the plates

were examined with an invertoscope for inhibition of cell growth. The medium was removed and 25 µL of a 5 mg/mL solution of MTT in phosphate-buffered saline was added to each well, the plates were incubated for 3 hours at 37°C. Subsequently, 125 µL of DMSO was added into each well to solubilize the formazan crystals (Johan *et al*, 2011) and shaken using an incubator shaker (New Brunswick Scientific: Excella E24 incubator Shaker Series) at 120 rpm for 15 minutes. The optical density was determined at 570 nm wavelength with an ELISA multiple well plate reader (Multiskan FC). The experiment was performed in triplicates. Cell viability (%) and percentage cytotoxicity for the extracts on the cell lines was calculated using the following equations (1, 2) (Senthilraja and Kathiresan, 2015).

$$\text{Cell viability (\%)} = \left(\frac{\text{Mean OD of treated}}{\text{Mean OD of control}} \times 100 \right) \quad (1)$$

$$\% \text{ cytotoxicity} = \left[\frac{(\text{Mean OD of control wells} - \text{Mean OD of treated wells})}{\text{Mean OD of control wells}} \right] \times 100 \quad (2)$$

Table 1. Percentage yield of methanol extracts

Plant Part Extracts	% Yield
Root	6.3
Stem Bark	2.5
leaves	3.6

Table 2. Qualitative Phytochemical Analysis

Phytochemicals	Leaves	Stem	Root
Steroids	-	+	+
Terpenoids	+	+	+
Saponins	+	++	+
Tannins	+	++	++
Flavonoids	+	++	++
Cardiac glycosides	-	-	-
Anthraquinone	+	+	+
Alkaloids	+	++	++

Key: - = absent, + = scanty, ++ = abundant

Data analysis

The data generated from the study were analysed using descriptive statistics and the cytotoxic concentration at 50% (CC₅₀) values determine using GraphPad prism.

3. Results

The result of the plant extraction is shown in **Table 1**. The root gave the highest extract yield with a value of 6.3% which is twofold that of the leaf (3.6%), and threefold the yield of the stem 2.5%. Phytochemicals such as steroids, terpenoids, saponins, tannins, flavonoids, cardiac glycosides, anthraquinones, and alkaloids were tested for in the three plant parts. The results showed that the three parts of *Mezoneuron benthamianum* contain six of the classes of phytochemicals as shown in **Table 2**. These phytochemicals were more abundant in the stem and the root extracts than in the leaves extract.

The quantitative analysis of certain phytochemicals in the different plant parts (**Fig. 1.**) shows that the root, stem and leaf had abundant quantities of flavonoids (67.2, 62.0 and 40.4%), the alkaloid content for root and stem was moderate (26.2 and 28.2%), while the leaf contained scanty amount (6.0%). Saponins were scanty in the root and leaf (2.6 and 5.8%), while stem had moderate quantity of 28.2 %.

Cytotoxicity effects

All the extracts caused 100% inhibition of growth of HEp-2 and HeLa cell line at concentrations of ≥ 50 $\mu\text{g/mL}$ as shown in Figures 1-3. At a concentration range of 3.125 - 6.25 $\mu\text{g/mL}$ methanol extract of *M. benthamianum* leaves and stem bark showed cytotoxic effects on HEp-2 cells, but not on HeLa cells (**Figures 2-3**).

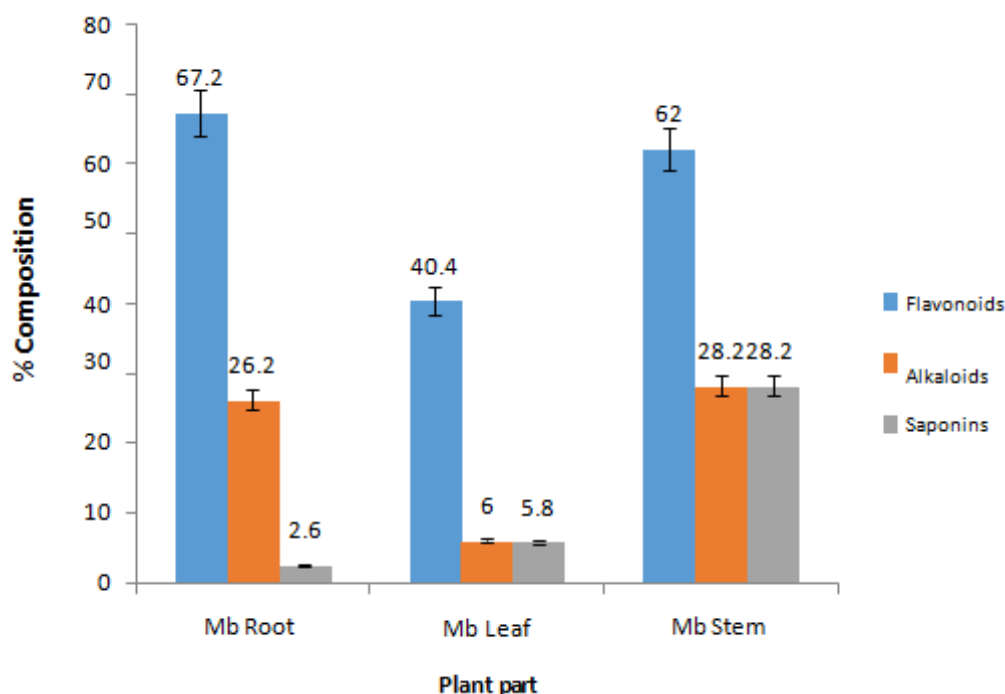


Fig 1. Quantitative Phytochemical constituents of the *M. benthamianum* parts
Abbreviations: **Mb Leaf** – *Mezoneuron benthamianum* leaf extract, **Mb Root** - *Mezoneuron benthamianum* root extract, **Mb Stem** - *Mezoneuron benthamianum* stem bark extract.

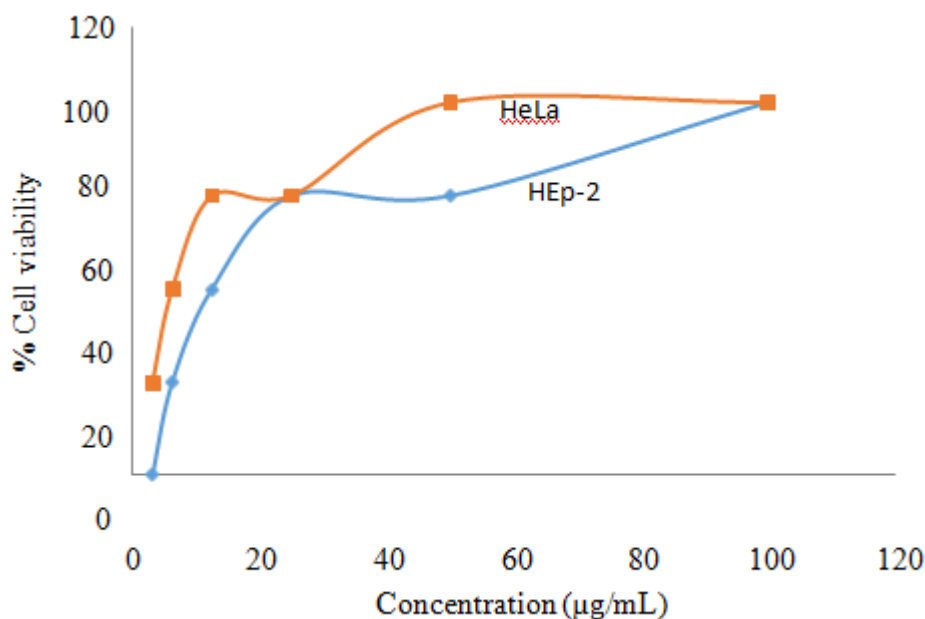


Fig. 2. Effects of root methanol extract of *M. benthamianum* viability of cancer cells

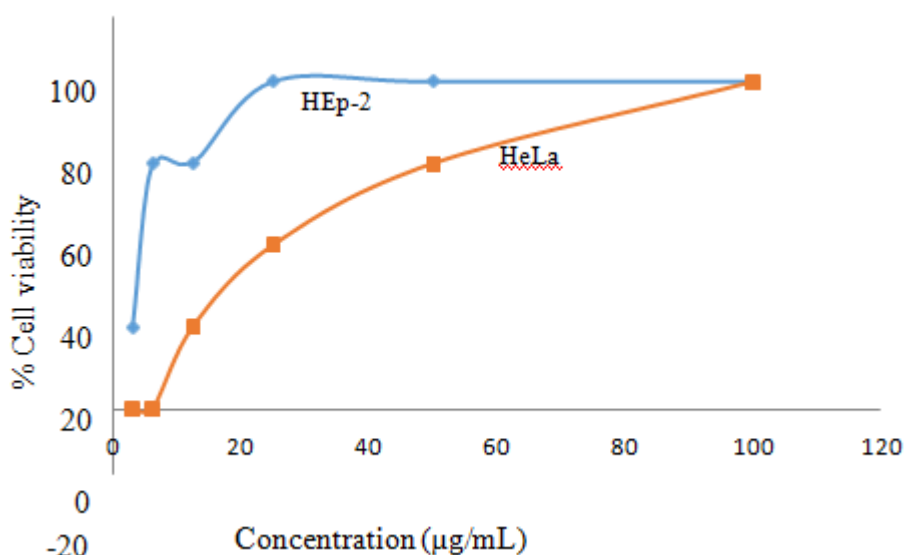


Fig. 3. Effects of leaf methanol extract of *M. benthamianum* viability of cancer cells

At 25 µg/mL, the cytotoxic effect of roots and leaves methanol extract on HEp-2 cells was 75% and 100%, respectively, and significantly ($p < 0.05$) higher compared to cytotoxic effect of stem bark.

The cytotoxic concentration (CC_{50}) of methanol extract of *Mezoneuron benthamianum* root on HeLa cells line was significantly ($p < 0.05$) lower compared to the CC_{50} of stem bark and leaves extracts (**Table**

3). The CC_{50} of methanol extract of *Mezoneuron benthamianum* leaves on HEp-2C cells line was significantly ($p < 0.05$) lower compared to the CC_{50} of stem bark and roots extracts. On Vero cells line, the methanol extract of *Mezoneuron benthamianum* leaves gave CC_{50} that was significantly ($p < 0.05$) higher compared to the CC_{50} of stem bark and roots extracts.

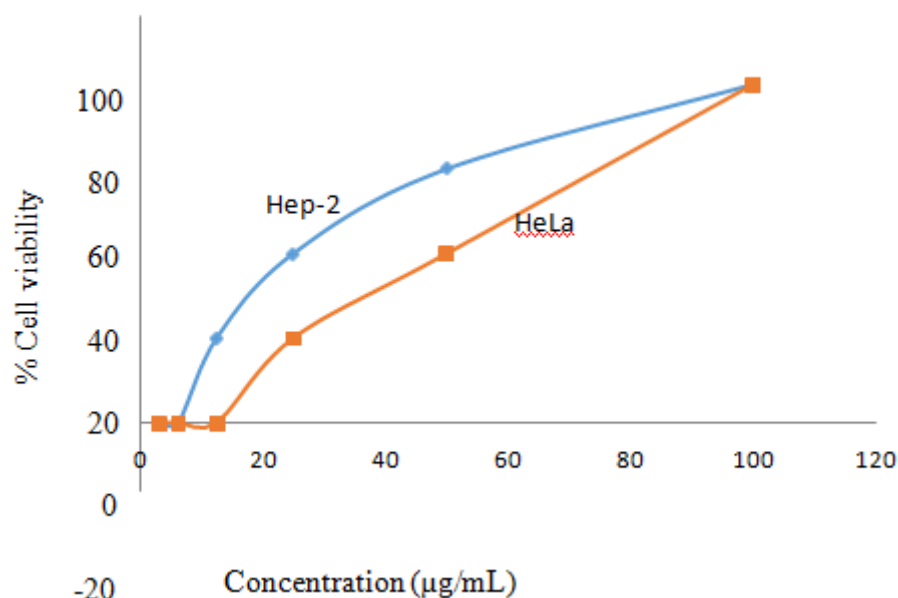


Fig. 4. Effects of stem bark methanol extract of *M. benthamianum* viability of cancer cells

Table 3. Cytotoxic concentration (CC₅₀) of methanol extracts on selected cells lines

Cell Lines	Extracts	CC ₅₀ (µg/mL)
HeLa	Mb Rt	15.64
	Mb Sb	34.20
	Mb Lf	23.06
HEp-2C	Mb Rt	21.23
	Mb Sb	23.26
	Mb Lf	11.38
Vero	Mb Rt	10.66
	Mb Sb	16.24
	Mb Lf	28.89

Abbreviations: Mb Lf= *Mezoneuron benthamianum* leaf; Mb Rt= *Mezoneuron benthamianum* root; Mb Sb= *Mezoneuron benthamianum* stem bark.

4. Discussion

Flavonoids, alkaloids, and saponins have been reported as phytochemicals that have cancer preventive activities against different types of cancer including estrogen-related cancers (Batra and Sharma, 2013; Isah, 2016; Shuli *et al.*, 2010). Flavonoids are a group of polyphenolic compounds possessing low molecular weight that exhibit a common benzo- γ -pyrone structure. They are categorized into various subclasses including flavones, flavonols, flavanones, isoflavanones,

isoflavanoids, anthocyanidins, and catechins (Hodnick *et al.*, 1988; Cook and Samman, 1996). The average human diet contains a considerable amount of flavonoids and the major dietary sources are fruits (orange, grapefruit, apple, and strawberry), vegetables (onion, broccoli, green pepper and tomato), soybeans and different herbs. Flavonoids have been shown to possess chemopreventive properties by Batra and Sharma (2013) and they have been reported to possess broad

spectrum of biological activities such as antioxidant, antibacterial and antiviral activities (Friedman, 2007; Cazarolli, 2008). Alkaloids have also been reported to have antitumor, antiviral and antibacterial activities etc. (Maomao *et al.*, 2015). Similarly, Isah (2016) reported that alkaloids had contributed immensely to the treatment of different types of cancers, while some saponins including ginsenosides and dioscin are reported to possess antitumor effects (Shuli *et al.*, 2010).

Qualitative test carried out on methanol extract of the stem, was positive for the presence of steroids, but not methanol extracts the leaves and root. All the extracts tested negative for the phytochemical group, cardiac glycosides. Results of phytochemical constituents of methanol leaves extract of *Mezoneuron benthamianum* obtained in this studies agrees with the report of Osho (2013). These findings are consistent with that of Osho (2013) who also reported the presence of these six secondary metabolites in the leaves of *Mezoneuron benthamianum*, and only differs in that the stem had a trace amount of steroids while cardiac glycosides were absent in all the plant parts. The high quantity of flavonoids in the three parts of the plant with moderate quantity of alkaloids particularly in the root and stem might explain the significant cytotoxic activity of the plant extracts. Flavonoids, alkaloids, and saponins have been reported to possess cancer preventive properties, and activity against different types of cancer including estrogen-related cancers (Batra and Sharma, 2013; Isah, 2016; Shuli *et al.*, 2010).

This study evaluated the cytotoxic potentials of the leaves, stems and roots methanol extracts of *Mezoneuron benthamianum* on some selected human cancer cell lines namely HeLa and HEP-2. In this study, the extracts exhibited concentration dependent cytotoxicity effects on the selected cancer cell lines with 100 µg/mL causing 100%

inhibition of cells growth. To corroborate these findings, Osamudiamen *et al.* (2017) previously reported the cytotoxic activities of compounds isolated from the root extract of *Mezoneuron benthamianum*. They isolated two cassane diterpenoids, taepenin A and nortaepeenin A, from the hexane extract of the roots of *Mezoneuron benthamianum*. Osamudiamen *et al.*, (2017) evaluated these compounds for *in vitro* cytotoxic activities against four cancer cells lines which include lung (NCI-H322), breast (T47D), prostate (PC-3) and lung (A549). They found out that the two compounds were significantly cytotoxic to the selected cancer cells lines.

Attempt was also made to investigate selectivity of the extracts using Vero cells line. Vero cell lines simulate normal human cell line and are often used in anticancer studies to investigate the selectivity of experimental anticancer agents. Results from this study shows that methanol root extract of *M. benthamianum* was cytotoxic to both HeLa and Vero cell lines ($CC_{50} = 10.66 \mu\text{g/mL}$) which is an indication of non-selective toxicity, therefore making it unacceptable as a prospective anticancer agent. The leaf extract exhibited more superior cytotoxic activity and selective for HEP-2 cells ($CC_{50} = 11.38 \mu\text{g/mL}$), compared to methanol extracts of the root and stem plant parts. The leaf extract thus demonstrated low cytotoxic activity on Vero given rise to $CC_{50} = 28.89 \mu\text{g/mL}$. This result suggests that *Mezoneuron benthamianum* leaf extract could be an excellent prospective candidate for cancer chemotherapy. It further suggests that different parts of the plants contain variable phytochemicals that exhibited anticancer activities relative to the cancer cell lines.

Conclusions

Methanol extracts of *Mezoneuron benthamianum* is rich in bioactive constituents and can be explored as a source of highly safe and effective alternative cancer chemotherapy in addition to currently available anticancer drugs.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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