Evaluation of extracts of wild Cannabis sativa L. for genotoxicity and phytochemical composition

Asita Okorie Asita*, Sibusisiwe Magama, Thato Mamoroesi Moahloli, Selometsi Baholo

Department of Biology, National University of Lesotho, P.O. Roma 180, Maseru 100, Lesotho, Southern Africa

Email address: aoasita@yahoo.co.uk, ao.asita@nul.ls; sibusisiwe.magama@gmail.com; kimanethato@gmail.com; solometsibaholo@gmail.com.

*Corresponding author.

Abstract. Cannabis sativa L. is used as medicine and narcotic in Lesotho. Phytochemical composition and total phenolics content (TPC) for hexane, chloroform, ethyl acetate and methanol extracts of aerial parts of C. sativa were determined. Ethyl acetate extract (0.1875, 0.375 and 0.75 mg mL⁻¹) and methanol extract (0.75, 1.5 and 3.0 mg mL⁻¹) were evaluated for cytotoxicity, genotoxicity and modulation of cyclophosphamide (CP, 1.25 mg mL⁻¹) and ethylmethane sulphonate (EMS, 0.25 mg mL⁻¹)-induced genotoxicity using Allium cepa root meristem assay. CP or EMS did not reduce mitotic index (MI) of cells, hence not cytotoxic when compared with negative control using the t-test (p>0.05), but genotoxic. Both extracts were genotoxic with methanol extract also being cytotoxic. Genotoxicity was the number of aberrant cells per 100 mitotic cells. Modulatory effect (ME) was obtained by comparing mutagen-induced genotoxicity with mixture-induced genotoxicity and expressed as the number of units of mutagen-induced genotoxicity that equalled the mixture-induced genotoxicity. ME was either positive or negative and significant only if ME = ≥ 2. Both extracts were genotoxic with methanol extract also being cytotoxic. Aberrations observed were sticky chromosomes, c-metaphase, anaphase and telophase bridges, chromosome fragments and laggards. Mixture of methanol extract with CP or EMS was more genotoxic (+ME range = 1.61-11.89) than the mutagen or extract alone which suggested synergistic interaction. Mixture of ethyl acetate extract with CP induced insignificant +ME. Mixture of ethylacetate extract with EMS was significantly more genotoxic (+ME = 2.20) than EMS only at high extract concentration. The methanol and ethylacetate extracts of C. sativa were not anti-genotoxic to CP- or EMS- induced genotoxicity. TPCs for hexane, chloroform, ethyl acetate and methanol extracts were 39831.46, 2544.94, 2438.20 and 56601.12 mg GAE/gram dry weight respectively. The differences in the cytotoxicity and MEs of the extracts were attributed to differences in phytochemical composition of extracts.

Keywords: medicinal cannabis, phenolics, modulatory effects, cyclophosphamide, ethyl methanesulphonate, Lesotho.
1. INTRODUCTION

Different human civilizations have depended for many centuries on plants and plant products for their medicinal (Balandrin et al. 1985) and recreational (Siegel 1977) needs.

The scientific basis for the use of plants in traditional medicine, has been attributed largely, to secondary metabolites (SMs) which have been shown to possess various biological activities (Bourgaud et al. 2001); therefore much of the protective and therapeutic effects of plants have been attributed to phytochemicals such as alkaloids, terpenoids, tannins, phenolics, etc. (Harborne 1998; Hertog et al. 1993; Zhang et al. 2001).

The concoctions used in traditional medicine are usually crude extracts in water, alcohol, distillates or essential oils, which contain many SMs from various structural groups and their activity is often due to synergistic interactions of the SMs present (Eid et al. 2012; Mulyaningish et al. 2010). At high concentrations, SMs change membrane fluidity and increase permeability. Therefore, many lipophilic SMs exhibit antimicrobial and cytotoxic activities and are responsible for the apparent broad-spectrum activity of concoctions used in traditional medicine (van Wyk and Wink 2015; Wink 2015).

In Lesotho, as in many other countries in the world, a system of traditional medicine based on the use of plants, birds, animals, their products and their combinations to treat a broad spectrum of communicable and noncommunicable diseases is still being practiced (Shale et al. 1999; Padmanabhan and Sujana, 2008). One plant species commonly used in traditional medicine in Lesotho and Southern Africa is Cannabis sativa (C. sativa), (Ranotsi et al. 2012). Other names for cannabis are marijuana, weed, dagga and “matekoane” in Sesotho (Ranotsi et al. 2012). Other names for cannabis are marijuana, weed, dagga and “matekoane” in Sesotho (Ranotsi et al. 2012; Bloomer 2019). This plant has been used for multiple purposes (medicinal, recreational, seed oil and industrial fiber, etc.) for thousands of years (ElSohly and Gul 2014). In Lesotho, C. sativa is used as medicine for all kinds of ailments such as heart burn, blood pressure and “nerves” as a recreational drug, and as part of religious rites (Laniel 1999).

A concoction of cannabis is a complex mixture of active compounds (phytochemicals) of which about 545 have been identified, 104 are cannabinoids or phytocannabinoids (as they originate from the plant) as well as 22 noncannabinoid constituents (Turner et al. 1980; ElSohly and Slade 2005; ElSohly and Gul, 2014). The cannabinoids include Δ9-tetrahydrocanabinol (THC), cannabidiol (CBD), cannabigerol (CBG), cannabichromenes (CBC), cannabinol (CBN) and cannabindiol (CBDL) (El-Alfy et al. 2010) found in the flowers, to a lesser extent the leaves, and minimally in the stems, and seeds (ElSohly and Gul 2014). THC is known as the major psychoactive component of cannabis that is responsible for causing addiction to marijuana (Ashton, 2001; National Institute on Drug Abuse (NIDA), 2018).

The importance of plants as sources of medicines not withstanding, investigations have revealed that many plants which are used as food or in traditional medicine have mutagenic, cytotoxic and genotoxic effects in vitro and in vivo assays (Higashimoto et al. 1993; Schimmeper et al. 1994; Kassie et al. 1996; Çelik and Aslantürk 2007). In a review by Marselos and Karamanakos (1999), they concluded that there was no consensus on the induction of point mutations by cannabinoids, while some experimental results suggest that cannabinoids may cause chromosomal damage (Zimmerman and Zimmerman 1990) and act as tumour promoters in animals. In addition, the extracts of some plant species have been observed to induce both mutagenic and antimutagenic effects on known mutagens in different test systems (Debisi et al. 1996).

The content of active compounds in plant species also vary according to their genetics, climatic factors, soil characteristics and the time of harvesting (Ramelet 2015); and when plant materials are extracted with solvents of different polarities, often the different solvent fractions contain different biomolecules (Herrera-Ruiz et al. 2008).

Studies on agents that modulate carcinogen-induced genotoxic effects in experimental animals provide endpoints that can be used for assessing the antimutagenic or anticarcinogenic properties of putative chemopreventive compounds and for predicting their protective efficacy in humans (Khaidakov et al. 2001).

In view of the foregoing therefore, the aim of this study was to evaluate hexane, methanol, ethyl acetate and chloroform extracts of wild Cannabis sativa for phytochemical composition, genotoxicity and the modulation of cyclophosphamide (CP)- and ethyl methanesulphonate (EMS)- induced genotoxicity using the Allium cepa chromosome aberration assay system.

The Allium cepa L assay is an in vivo assay and one of the established plant bioassays, validated by the international programme on chemical safety (IPCS, WHO), as an efficient and standard test for chemicals screening, in situ monitoring of the genotoxicity of environmental substances (Leme and Marin-Morales 2009) and to evaluate the genotoxic potential of medicinal plants (Camparoto et al. 2002; Knoll et al. 2006; Fachinetti et al. 2007; Lubini et al. 2008; Fachinetti et al. 2009). The Allium cepa L assay tests genotoxicity using chromosomes and therefore detects chromosome structural and numerical alterations (Tedesco and Laughinghouse 2012; Bonciu et al. 2010).
Evaluation of extracts of wild Cannabis sativa L. for genotoxicity and phytochemical composition

CP is an antineoplastic indirect-acting (promutagen) alkylating agent (Mohn and Ellenberger 1976; Hales 1982) while EMS is a direct-acting mutagen, tetratogen, and brain carcinogen (Stubbs et al. 1997).

2. MATERIALS AND METHODS

2.1 Test organism

Onion (A. cepa) seeds of the variety, Texas Grano 502 P.R.R., a product of Sakata seeds, Lanseria 1748, Republic of South Africa were purchased from Maseru Garden Centre, Lesotho in Southern Africa.

2.2 Mutagens and chemicals

Cyclophosphamide (CP) and ethyl methanesulfonate (EMS) are products of Fluka (Biochemika, Germany). Methanol (absolute) is a product of Associated Chemical Enterprises (Pty) Ltd (Johannesburg, South Africa); hydrochloric acid glacial and acetic acid are products of UNILAB (Krugerdorp, South Africa); acetocarmine stain was obtained from Carolina Biological Supply Company, Burlington, North Carolina, USA.

2.3 Plant material collection and preparation

Aerial parts of the female plant of wild C. sativa were collected from the Thaba Bosiu area, some 12 km from the National University of Lesotho (NUL) campus, in the Maseru District of Lesotho where they grow in a location with the following geographical coordinates: Latitude: 29°22’49”S, Longitude: 27°33’13” E and at an altitude of 1600 m. The aerial parts of the sample were dried in a fanned Labcon oven at 37°C to a constant weight and brittle, about 48 hours. Thereafter, the pieces were segmented and ground to a fine powder using a pulveriser (Kenwood) and the powder was stored in sealed amber bottles in the dark at room temperature.

2.4 Preparation of the crude C. sativa extracts

Sequential solvent extraction of the ground powder was done according to the method outlined in Razak et al. (2014); Padhi and Panda (2015); Fayera et al. (2018) with slight modifications. All crude extracts (hexane, chloroform, ethyl acetate and methanol) were stored at 4°C until further investigation for genotoxicity, modulatory effects on mutagen-induced genotoxicity and phytochemical profiling.

2.5 Qualitative Phytochemical Screening of crude extracts of C. sativa

The crude extracts of C. sativa prepared with hexane, chloroform, ethyl acetate and methanol were subjected to a qualitative screening for the presence of major phytochemical classes using standard phytochemical methods and the appropriate reagents and chemicals according to the modified methods of Trease and Evans (1984); Trease and Evans (2002); Soni and Sheetal (2013); Nwaoguikpe et al. (2014) and Uddin et al. (2014). Each reaction mixture was visually assessed as in Lu et al. (2014), for precipitate formation, foam formation, colour changes and colour intensity according to the following key: (+), Low intensity of colour and/or precipitate; (++), moderate intensity of colour and/or precipitate; (+++), strong intensity of colour and/or precipitate (-), not detected (either absent or below the detection limit).

The list of screening tests that were carried out is shown in Table 1.

2.6 Determination of the total phenolic content of C. sativa extracts using the Folin-Ciocalteau assay

Determination of the total phenolic content for each of the extracts was done by the method of McDonald

| Table 1. Phytochemical screening tests. |
|----------------------------------------|
| Phytochemical | Name of test | Colour for positive test |
|---------------|--------------|--------------------------|
| 1. Flavonoids | Shinoda      | Pink                     |
| 2. Alkaloids  | Wagner       | Blue-black/Blue-black    |
| 3. Tannins    | Ferric chloride | Reddish-brown            |
| 4. Terpenoids | Salkowski    | Foam formation           |
| 5. Saponins   | Foam test    | Green                    |
| 6. Simple phenols | Ferric chloride | Blue                  |
| 7. Polyphenols | Ferric chloride | Blue-green               |
| 8. Anthocyanins | NaOH      | Red precipitate          |
| Betacyanins   | NaOH         | Yellow                   |
| Quinones      | HCl          | Green                    |
| Phlobatannins | HCl          | Red precipitate          |
| Anthraquinones | HCl+chloroform+ammonia | Rose-pink/violet      |
| Coumarins     | NaOH+chloroform | Yellow                  |
| Phytosterols  | Salkowski    | Red                      |
| Cardiac glycosides and Cardenolides | Keller-Kiliian’s | Brown-red ring |
| Reducing sugars | Benedict’s | Red precipitate          |
| Proteins      | Biuret       | Violet                   |
| Amino acids   | Ninhydrin    | purple                   |
| Fatty acids   | Diethyl ether | Transparent stain        |
et al. (2001) with slight modifications. The total phenolic content of each extract was recorded in milligram gallic acid equivalents (GAE) per gram of dry weight of extract from the gallic acid standard curve (Wong et al., 2012; Moyo et al. 2013; Magama et al., 2018). The total phenolic content in each extract determined as milligram gallic acid equivalents (GAE) per gram of dry weight of extract was calculated using the following formula:

\[ T = \frac{(C \times V)}{M} \]  

(1)

Where \( T \) is the total phenolic content of the extracts in mg GAE per gram of dry weight of extract, \( C \) is the concentration of the gallic acid established from the calibration curve in mg mL\(^{-1}\), \( V \) is the volume of extract in mL and \( M \) is the mass of the plant extract in g.

2.7 Genotoxicity Experiments

The preliminary assay to select concentrations of mutagens and plant extracts to use and Genotoxicity assay (including the treatment of Allium cepa seedlings with test agents, root harvest, slide preparation and scoring of slides) were conducted according to the methods of Asita et al. (2017). Due to insolubility of the hexane and chloroform extracts of C. sativa only the methanol and ethyl acetate extracts were evaluated for genotoxicity using 2.5% acetone (v/v in distilled water) as the solvent. The 2.5% acetone was not toxic or genotoxic to the onion root meristem cells. From the results of the preliminary assays to select the concentrations of mutagens and plant extracts to use, the following concentrations of plant extracts (in mg mL\(^{-1}\)); methanol extract (0.75, 1.5 and 3.00) and ethyl acetate extracts (0.750, 0.375, 0.1875 and 0.09375); CP (1.25.00) and EMS (0.250) were assessed for cytotoxicity that is, mitotic index (MI), genotoxicity (GT) and the modulatory effect (ME) of plant extracts on mutagen-induced genotoxicity.

The aberrations assessed were: sticky chromosomes (S), C-metaphase (C-Mit), lagging chromosomes (L), chromosome bridges at anaphase and telophase (A.B) and chromosome fragment (F). For calculating the GT, only aberrant mitotic cells were considered.

2.8 Analysis of slide preparations

2.8.1 Cytotoxicity

The mitotic index (MI) was expressed as the number of dividing cells per 100 cells scored according to the formula:

\[ \text{MI} = \frac{\text{Number of dividing cells}}{100} \times \frac{1}{\text{Total number of cells scored}} \]  

(2)

The MI was used as a measure of cytotoxicity (CT). The MI of each treatment group was compared with that of the negative control group using t-test at a probability level of 0.05, using the SPSS for windows, version 11.0 software.

2.8.2 Genotoxicity

Genotoxicity (GT) was expressed as the number of aberrant mitotic cells (AMC) per 100 mitotic cells \([\text{i.e.} \text{AMC + normal mitotic cells (NMC)}]\) scored according to the formula:

\[ \text{Frequency of GT} = \frac{\text{AMC}}{\text{AMC + NMC}} \times 100 \]  

(3)

The mean GT of each group of three slides per concentration of test agent was compared with that of the negative control group using t-test. P values less than 0.05 (P < 0.05) were considered as indicative of significance.

2.8.3 Modulatory effect (ME) of plant extracts on mutagen-induced genotoxicity

The modulatory effect (ME) of plant extract on CP- or EMS-induced genotoxicity (GT) was calculated using the formula of Asita et al. (2017):

\[ \text{ME} = \frac{(B - C) - (A - C)}{A - C} \]  

(4)

Where ‘A’ is the genotoxicity induced by the mutagen (CP or EMS) alone, i.e. mutagen-induced genotoxicity; ‘B’ is the genotoxicity induced by mixture of plant extract and mutagen, i.e. mixture-induced genotoxicity and ‘C’ is the genotoxicity induced by negative control, such as tap water alone.

The modulatory effect (ME) was thus obtained by comparing the mutagen-induced genotoxicity (A) with the mixture-induced genotoxicity (B). The ME value indicated the number of units of the mutagen-induced genotoxicity (A) that equaled the mixture-induced genotoxicity (B). ME was significant only if ME was ≥ 2, i.e mixture was at least twice (200%) more (+) or less (-) genotoxic than mutagen alone.

A positive ME (+ME) indicated that the mixture was more genotoxic (increased GT) than the mutagen and if mixture is also more genotoxic than the genotoxic plant extract alone then a synergistic interaction is inferred.
But mutagen-potentiation is inferred if mixture is less genotoxic than the non-genotoxic plant extract alone.

A negative ME (-ME) indicated that the mixture was less genotoxic (reduced GT) than the mutagen alone. If mixture is less genotoxic than the mutagen and the genotoxic plant extract then antagonism is inferred. However, if mixture is less genotoxic than mutagen and also, more- or less genotoxic than the non-genotoxic plant extract then it is antimutagenicity.

2.8.4 Data analysis

In the determination of total phenolics content, data was expressed as means ± standard deviations of three replicate determinations using Microsoft excel 2016. Differences between controls and treatment groups were determined using Student’s t-test. P-values of less than 0.05 (p<0.05) were considered statistically significant using the IBMSPSS statistics, version 20 software. Regression equations and graphs were used for the determination of milligram gallic acid equivalents (mgGAE equivalents) per gram of dry extract and the concentration of extract needed to inhibit oxidation by 50% (IC50). For the genotoxicity assays, the mean value of each group of three slides per concentration of test agent was compared with that of the negative (solvent) control group using student’s t-test and the Chi square test. P-values less than 0.05 (p < 0.05) were considered as indicative of significance.

3. RESULTS

3.1 Qualitative biochemical profile of C. sativa solvent extracts

In Table 2 is presented the qualitative phytochemical profile of different solvent extracts of C. sativa obtained from the various tests. The methanol extract contained the highest number of the different phytochemical classes (15/19), followed by hexane and chloroform (9/15 each) and ethyl acetate (7/15). Polyphenols as a class and the polyphenols (namely- anthocyanins, betacyanins, coumarins, and flavonoids), quinones (aromatic ketones), simple phenols were detected in trace amounts in the hexane, chloroform and methanol extracts but not detected in the ethylacetate extract. Coumarins (also polyphenols) were present in all extracts, though in traces only in the ethyl acetate extract. Traces of amino acids were also detected in all the solvent extracts. Terpenoids were detected only in the hexane and methanol extracts. Flavonoids (also polyphenolic), alkaloids, sapo-

nins (steroid and terpenoid glycosides) and phlobatannins (also polyphenolic), were detected in the methanol extract only. Phytosterols (unsaturated steroid alcohols) were detected only in the hexane extract. Cardiac glycosides and cardenolides, proteins and fatty acids were not detected in any of the solvent extractives.

3.2 Quantitative determination of total phenolics content

In Fig. 1, is presented the gallic acid calibration curve for determination of total phenolic content of the different solvents (hexane, chloroform, ethyl acetate and methanol) extracts, where y was the mean absorbance of the sample at 760nm and x the concentration established from the gallic acid calibration curve. The regression equation was $y = 0.0712x - 0.1055$; the total phenolic content of the hexane extract, $x$, with $y = 0.0363$ was found to be 39 831.46mg GAE/gram dry weight. The total phenolic content of the chloroform extract, $x$, with $y = 0.0757$ was 2544.94 mg GAE/gram dry weight. The total phenolic content of the ethyl acetate extract, $x$, with $y = 0.0681$ was 2438.20mg GAE/gram dry weight. The

| Test                | Hexane | Chloroform | Ethylacetate | Methanol (95%) |
|---------------------|--------|------------|--------------|----------------|
| Flavonoids          | -      | -          | -            | ++             |
| Alkaloids           | -      | -          | -            | +++            |
| Tannins             | -      | +          | -            | +++            |
| Terpenoids          | +++    | -          | -            | +++            |
| Saponins            | -      | -          | -            | +              |
| Simple phenols      | +      | +          | -            | +              |
| Polyphenols         | +++    | +++        | +++          | +++            |
| Anthocyanins        | +++    | +++        | +            | +++            |
| Betacyanins         | +++    | +          | +            | +              |
| Quinones            | +++    | +++        | +++          | +++            |
| Phlobatannins       | -      | -          | -            | +              |
| Anthraquinones      | -      | -          | -            | +              |
| Coumarins           | ++     | +          | +            | ++             |
| Phytosterols        | +++    | -          | -            | -              |
| Cardiac glycosides  | -      | -          | -            | -              |
| and Cardenolides    |        |            |              |                |
| Reducing sugars     | -      | +          | ++           | +++            |
| Proteins            | -      | -          | -            | -              |
| Amino acids         | +      | ++         | ++           | +++            |
| Fatty acids         | -      | -          | -            | -              |

Key: Low intensity = “+”; moderate intensity = “++”; strong intensity = “+++”; not detected or negative = “-“.(Lu et al., 2014).
total phenolic content of the methanol extract, \( x \), with \( y = 0.0960 \) was 56 601.12 mg GAE/gram dry weight.

### 3.3 Cytotoxicity and Genotoxicity

#### 3.3.1 Figures and Tables

Photographs of the most representative pictures of normal mitotic cells and cells containing the different types of chromosome aberrations that were observed and scored are presented in Figure 2. The results of the cytotoxicity and genotoxicity experiments with the methanol and ethyl acetate extracts of *C. sativa* are presented in Tables 3 and 4 respectively.

**Figure 1.** Gallic acid calibration curve for determination of Total phenolic content for hexane and methanol extracts.

**Figure 2.** Photomicrographs of cells of *Allium cepa* showing untreated cells in normal division stages and Chromosomal aberrations (arrowed) in cells treated with methanol and ethyl acetate extracts of *Cannabis sativa* or mixture of extracts with EMS or Cyclophosphamide. (a) Interphase (b) Normal Prophase (c) Normal metaphase (d) Normal metaphase (e) Early anaphase (f) Late anaphase (g) Telophase (h) Pyknotic interphase nuclei with micronucleus (i) Prophase with sticky chromosomes (j) Metaphase with sticky chromosomes (k) Metaphase with sticky chromosomes (l) C-metaphase (m) Metaphase with dislocated chromosome (n) Late anaphase with dislocated chromosome (o) Anaphase with sticky and scattered chromosomes (p) Late anaphase with chromosome bridge (q) Telophase with lagging chromosome (r) Telophase with sticky chromosomes and bridge (s) telophase with chromosome bridge and lagging (t) telophase with chromosome bridge and fragment (u) Telophase with chromosome bridge (v) Telophase with chromosome fragment and lagging. Magnification is 1000 X.
3.3.2 Results for methanol (95%) extract experiments in Table 3

In Table 3 are the results of cytotoxicity and genotoxicity experiments with methanol (95%) extracts of *C. sativa* and the mutagens, CP and EMS.

(P+M)/(A+T) Ratio: Examination of the (P+M)/(A+T) ratio in column 8 of Table 3 shows that only the treatment with the lowest concentration (0.75 mg/mL) of *C. sativa* extract alone or in a mixture with EMS (0.75 mg/mL) induced a significant change in (P+M)/(A+T) ratio, when compared with the solvent (2.5% acetone) treated negative control group ($p < 0.05$).

Cytotoxicity: Examination of the MI in column 9 of Table 3 shows that CP (1.25 mg mL$^{-1}$) and EMS (0.25 mg mL$^{-1}$) were not toxic. All the concentrations of the methanol extract of *C. sativa* (0.75, 1.5, 3.0 mg/mL) and their mixtures with CP (1.25 mg mL$^{-1}$) or EMS (0.25 mg mL$^{-1}$) induced significant reduction of the MI (was toxic) when compared to the solvent (2.5% acetone) treated negative control ($p<0.05$).

Genotoxicity (GT): Examination of induction of genotoxicity in column 10 of Table 3 shows that CP (1.25 mg mL$^{-1}$), EMS (0.25 mg mL$^{-1}$), methanol extract (0.75, 1.5, 3.0 mg/mL) and the mixtures of CP (1.25 mg mL$^{-1}$) or EMS (0.25 mg mL$^{-1}$) with each concentration of *C. sativa* Table 3.

### Table 3. Cytotoxic and genotoxic effects of methanol extracts of *C. sativa*, EMS and CP on meristem cells of onion root tip and the modulatory effects (ME) of the methanol extract of *C. sativa* on EMS- or CP-induced genotoxicity.

| Treatment (TC in mg/mL) | Conc. | Inter. Cells | Total number of cells in the stages of mitosis in 2000 cells scored | Total number of cells scored | (P+M)/(A+T) | MI | Genotoxicity | Modulatory Effect |
|------------------------|-------|--------------|---------------------------------------------------------------|-----------------------------|-----------|----|-------------|------------------|
|                        |       |              | $N$ $ABN$ $Total$ $N$ $+ ABN$ $2000$ $2.60$ $10.50$ $1.05$ | $2.28^*$ $2.28^*$ $24.78^*$ |           |    |             |                  |
| Acetone (2.5%)          | MEAN  | 1790.00      | 208.00 $2.00$ 210.00 $2000$ 2.60 $10.50$ $1.05$          | $75.54$ $75.54$ $75.54$ $75.54$ $75.54$ $0.00$ $0.09$ $3.78$ |           |    |             |                  |
| CP (1.25)               | MEAN  | 1856.00      | 135.33 $8.67$ 144.00 $2000$ 2.56 $7.20$ $7.31^*$         | $48.91$ $6.11$ $44.80$ $0.00$ $0.33$ $2.24$ $7.06$ |           |    |             |                  |
| EMS (0.25)              | MEAN  | 1815.33      | 171.00 $13.67$ 184.67 $2000$ 3.86 $9.23$ $5.50^*$          | $99.36$ $16.50$ $99.36$ $0.00$ $1.60$ $4.97$ $6.42$ |           |    |             |                  |
| *C. sativa* (0.75)      | MEAN  | 1954.33      | 34.33 $11.33$ 45.67 $2000$ 8.87 $2.28^*$ $24.78^*$         | $5.53$ $1.15$ $5.53$ $0.00$ $0.99$ $0.08$ $1.80$ |           |    |             |                  |
| *C. sativa* (1.5)       | MEAN  | 1984.33      | 11.33 $4.33$ 15.67 $2000$ 3.47 $0.78^*$ $27.58^*$          | $1.53$ $0.58$ $1.53$ $0.00$ $0.92$ $0.06$ $1.58$ |           |    |             |                  |
| *C. sativa* (3.0)       | MEAN  | 1942.00      | 39.67 $18.33$ 58.00 $2000$ 3.59 $2.90^*$ $31.17^*$         | $16.09$ $6.51$ $16.09$ $0.00$ $2.16$ $0.80$ $2.69$ |           |    |             |                  |
| *C. sativa* (0.75) + CP | MEAN  | 1989.00      | 2.33 $6.67$ 8.00 $11.00$ $2000$ 2.50 $0.55^*$ $77.91^*$ | $2.00$ $1.53$ $2.89$ $0.00$ $0.90$ $0.10$ $14.34$ |           |    |             |                  |
| *C. sativa* (1.5) + CP  | MEAN  | 1991.00      | 1.67 $7.33$ 9.00 $2000$ 4.67 $0.45^*$ $81.76^*$          | $1.00$ $0.58$ $1.00$ $0.00$ $2.31$ $0.05$ $5.09$ |           |    |             |                  |
| *C. sativa* (3.0) + CP  | MEAN  | 1981.00      | 10.00 $9.00$ 19.00 $2000$ 3.88 $0.95^*$ $47.59^*$         | $4.00$ $2.65$ $1.73$ $4.00$ $0.00$ $2.51$ $0.20$ $4.65$ |           |    |             |                  |
| *C. sativa* (0.75) + EMS| MEAN  | 1959.00      | 24.00 $17.00$ 41.00 $2000$ 4.81 $2.05^*$ $41.55^*$        | $12.49$ $12.49$ $12.49$ $0.00$ $0.97$ $0.62$ $4.76$ |           |    |             |                  |
| *C. sativa* (1.5) + EMS | MEAN  | 1950.00      | 43.67 $6.33$ 50.00 $2000$ 2.83 $2.50^*$ $12.67^*$        | $0.00$ $0.58$ $0.58$ $0.00$ $0.54$ $0.00$ $1.15$ |           |    |             |                  |
| *C. sativa* (3.0) + EMS | MEAN  | 1964.33      | 30.33 $5.33$ 35.67 $2000$ 3.14 $1.78^*$ $15.00^*$        | $15.89$ $13.58$ $2.31$ $15.89$ $0.00$ $0.48$ $0.79$ $0.33$ |           |    |             |                  |

Key: TC = Test compound; N = Normal mitotic cells (comprising prophase, metaphase, anaphase and telophase); ABN = Aberrant mitotic cells; SD = Standard deviation; CP = Cyclophosphamide; EMS = Ethylmethane sulphonate; Cs = *Cannabis sativa*; MI = Mitotic index; J = $P+M/A+T$ ratio (significant increase in ratio compared to negative control, $P<0.05$ in the t-test, $n=3$); * = TC is Toxic (MI treatment significantly different from negative control, $P<0.05$ in the t-test, $n=3$); # = TC is genotoxic (significant difference from negative control, $P<0.05$ in the t-test, $n=3$); † = *C. sativa* + Mutagen mixture more genotoxic than mutagen alone (Synergism); ‡ = *C. sativa* + Mutagen mixture less genotoxic than mutagen or *C. sativa* alone (Antagonism); †† = *C. sativa* + Mutagen mixture more genotoxic than mutagen alone but less than *C. sativa* alone; ‡‡ = *C. sativa* + Mutagen mixture less genotoxic than mutagen alone (Antimutagenicity) but more than *C. sativa* alone.
extract used were all genotoxic to the root meristem cells of *A. cepa* when compared to the solvent (2.5% acetone) treated negative control (*P* < 0.05).

**Modulatory effect (ME) of methanol (95%) extract of *C. sativa* on CP or EMS-induced Genotoxicity (GT):**

Examination of the modulatory effect (ME) in column 11 of Table 3 shows that the mixture of each of the three concentrations of *C. sativa* (0.75, 1.5 or 3.0 mg/mL) methanol extract with CP (1.25 mgmL⁻¹) was significantly (> twofold or 200%) more genotoxic than CP or *C. sativa* extract alone. The mixture of each concentration of *C. sativa* extract with CP thus induced a positive and significant (> twofold) value of ME (11.27, 11.89 and 6.43 respectively) and synergism or synergistic interaction between the *C. sativa* extracts and CP, was inferred.

Examination of the modulatory effect (ME) in column 12 of Table 3 shows that the mixture of each of the three concentrations of *C. sativa* (0.75, 1.5 or 3.0 mg/mL) methanol extract with EMS (0.25 mg/mL) was more genotoxic than EMS alone. The mixture of *C. sativa* (0.75 mg/mL) was also more genotoxic than *C. sativa* extract alone. The mixtures of *C. sativa* (0.75 or 3.00 mg/mL) extract with EMS induced a positive and significant (> twofold) value of ME (8.10 and 2.14 respectively) and

---

**Table 4. Cytotoxic and Genotoxic effects of ethylacetate extracts of *C. sativa*, EMS and CP on meristem cells of onion root tip and the modulatory effects (ME) of the ethylacetate extract of *C. sativa* on EMS- or CP-induced genotoxicity.**

| Treatment (TC in mg/mL) | Conc. | Inter. Cells | Total number of cells in the stages of mitosis in 2000 cells scored | Total number of cells scored (P+M)/(A+T) MI Genotoxicity | Modulatory Effect Extract on CP Extract on EMS |
|-------------------------|-------|--------------|---------------------------------------------------------------|------------------------------------------------------------|-------------------------------------------------|
| Acetone (2.5%)          | MEAN  | 1790.00      | 208.00 2010.00                                               | 2.60 10.50 1.05                                           |                                                 |
|                         | SD    | 75.54        | 75.54 75.54 0.00                                             | 0.09 3.78 0.41                                           |                                                 |
| CP (1.25)               | MEAN  | 1856.00      | 135.33 144.00                                               | 2.56 7.20 0.73                                           |                                                 |
|                         | SD    | 44.80        | 48.91 6.11 44.80                                            | 0.03 0.33 0.24                                           |                                                 |
| EMS (0.25)              | MEAN  | 1815.33      | 171.00 184.67                                               | 3.86 9.23 5.50                                           |                                                 |
|                         | SD    | 99.36        | 86.63 16.50 99.36                                           | 0.00 1.60 4.97                                           |                                                 |
| *C. sativa* (0.1875)    | MEAN  | 1828.67      | 93.67 171.33                                               | 6.58 J 8.57 45.72                                        |                                                 |
|                         | SD    | 21.13        | 22.03 11.59 21.13                                           | 0.00 0.61 1.06                                           |                                                 |
| *C. sativa* (0.375)     | MEAN  | 1805.00      | 180.67 195.00                                               | 2.28 9.75 7.01                                           |                                                 |
|                         | SD    | 21.00        | 11.59 11.59 21.00                                           | 0.00 0.55 1.05                                           |                                                 |
| *C. sativa* (0.75)      | MEAN  | 1824.67      | 171.00 175.33                                               | 3.17 8.77 2.37                                           |                                                 |
|                         | SD    | 39.63        | 37.59 2.08 39.63                                            | 0.00 1.55 1.98                                           |                                                 |
| *C. sativa* (0.1875) + CP | MEAN  | 1786.33      | 190.67 213.67                                               | 2.87 10.68 10.26                                         | 0.47†                                            |
|                         | SD    | 86.05        | 74.51 12.29 86.05                                           | 0.00 0.74 4.30                                           |                                                 |
| *C. sativa* (0.375) + CP | MEAN  | 1862.33      | 133.33 137.67                                               | 3.12 6.88 4.16                                          | -0.50‡                                           |
|                         | SD    | 50.96        | 53.35 2.52 50.96                                            | 0.00 1.79 2.55                                           |                                                 |
| *C. sativa* (0.75) + CP | MEAN  | 1817.00      | 178.00 183.00                                               | 3.50 9.15 2.75                                          | -0.73‡                                           |
|                         | SD    | 20.66        | 20.42 1.00 20.66                                            | 0.00 1.18 1.03                                           |                                                 |
| *C. sativa* (0.1875) + EMS | MEAN  | 1807.00      | 189.33 193.00                                               | 3.09 9.65 1.88                                          | -0.81‡                                           |
|                         | SD    | 23.64        | 22.85 2.08 23.64                                            | 0.00 0.91 1.18                                           |                                                 |
| *C. sativa* (0.375) + EMS | MEAN  | 1820.67      | 174.67 179.33                                               | 2.32 8.97 2.67                                          | -0.64‡                                           |
|                         | SD    | 33.01        | 33.31 1.15 33.01                                            | 0.00 0.45 1.65                                           |                                                 |
| *C. sativa* (0.75) + EMS | MEAN  | 1898.33      | 99.33 101.67                                               | 3.27 5.08 15.28                                        | 2.20†                                            |
|                         | SD    | 94.50        | 95.00 58.40 94.50                                           | 0.00 0.67 4.73                                          | 23.89                                            |

TC = Test compound; N = Normal mitotic cells (comprising prophase, metaphase, anaphase and telophase); ABN = Aberrant mitotic cells; SD = Standard deviation; CP = Cyclophosphamide; EMS = Ethylmethane sulphonate; *C.s = Cannabis sativa; MI = Mitotic index; J = P+M/A+T ratio (significant increase in ratio compared to negative control, *P* < 0.05 in the t-test, *n* = 3); * = TC is Toxic (MI treatment significantly different from negative control, *P* < 0.05 in the t-test, *n* = 3); # = TC is genotoxic (significant difference from negative control, *P* < 0.05 in the t-test, *n* = 3); † = *C.s* + Mutagen mixture more genotoxic than mutagen or *C.s* alone (Synergism); ‡ = *C.s* + Mutagen mixture less genotoxic than mutagen or *C.s* alone (antagonism); †† = *C.s* + Mutagen mixture more genotoxic than mutagen alone but less than *C.s* alone; ‡‡ = *C.s* + Mutagen mixture less genotoxic than mutagen alone (antimutagenicity) but more than *C.s* alone.
synergism or synergistic interaction between the *C. sativa* extracts and EMS at those concentrations were inferred.

3.3.3 Results for ethylacetate extract experiments in Table 4

In Table 4 are the results of cytotoxicity and genotoxicity experiments with ethylacetate extracts of *C. sativa* and the mutagens, CP and EMS.

**Examination of the (P+M)/(A+T) Ratio:** Examination of the (P+M)/(A+T) ratio in column 8 of Table 4 shows that only the treatment with the lowest concentration (0.1875 mg/mL) of *C. sativa* extract induced a significant change in (P+M)/(A+T) ratio, when compared with the solvent (2.5% acetone) treated negative control group (P<0.05).

**Cytotoxicity:** Examination of the MI in column 9 of Table 4 shows that none of the treatments induced a reduction of the MI when compared to the solvent (2.5% acetone) treated negative control (P<0.05) and were adjudged none toxic to the root meristem cells of *A. cepa* i.e. CP (1.25 mg mL\(^{-1}\)), EMS (0.25 mg mL\(^{-1}\)) and all concentrations of the ethylacetate extract of *C. sativa* (0.1875, 0.375, 0.75 mg/mL) and the mixtures of the individual concentrations of the *C. sativa* extract with CP (1.25 mg mL\(^{-1}\)) or EMS (0.25 mg mL\(^{-1}\)) were all none toxic.

**Genotoxicity (GT):** Examination of induction of genotoxicity in column 10 of Table 4 shows that CP (1.25 mg mL\(^{-1}\)) and EMS (0.25 mg mL\(^{-1}\)) were genotoxic to the root meristem cells of *A. cepa* when compared to the solvent (2.5% acetone) treated negative control (P<0.05). The two lowest concentrations of ethylacetate extracts of *C. sativa* (0.1875 and 0.375 mg mL\(^{-1}\)) and EMS (0.25 mg mL\(^{-1}\)) were also genotoxic. The mixture of each concentration (0.1875, 0.375 or 0.75 mg/mL) of *C. sativa* extract with CP (1.25 mg mL\(^{-1}\)) was also genotoxic. The mixture of each concentration of the two higher concentrations (0.375 or 0.75 mg/mL) of *C. sativa* extract with EMS was also genotoxic, but not the mixture of the lowest concentration (0.1875 mg/mL) with EMS.

**Modulatory effect (ME) of ethylacetate extract of *C. sativa* on CP or EMS-induced Genotoxicity (GT):**

Examination of the modulatory effect (ME) in column 11 of Table 4 shows that the mixture of the lowest concentration of the ethylacetate extract of *C. sativa* (0.1875 mg/mL) with CP (0.25 mg/mL) was none significantly (< twofold, ME = -0.81 and -0.64 respectively) less genotoxic than EMS or *C.s* extract alone. However the mixture of the highest concentration of *C.s* extract (0.75 mg/mL) with EMS was significantly (> twofold (200%), ME = 2.20) more genotoxic than EMS or *C.s* extract alone, and synergism or synergistic interaction between the EMS and *C. sativa* extract, at that concentration, was inferred.

4. DISCUSSION

In this study, hexane, chloroform, ethyl acetate and methanol extracts of the aerial parts of *Cannabis sativa* (*C. sativa*) growing in Lesotho and used in traditional medicine to treat some diseases and for recreational purposes were evaluated for phytochemical composition, genotoxicity and modulatory effects on EMS- and CP – induced genotoxicity using the onion (*Allium cepa* L.) chromosome aberration assay system.

The results of the phytochemical screening tests are presented in Table 2 while the results of the genotoxicity tests are presented in Tables 3 and 4.

In the present qualitative study presented in Table 2, based on the intensity of the colour in the colorimetric tests and (or) the appearance of precipitates, during the identification reactions, methanol extract contained the highest number of the different phytochemical classes (15/19) followed by hexane and chloroform (9/19 each) and ethylacetate (7/19). Such tests allow a semi-quantitative evaluation for the presence of secondary metabolites in extract solutions (Chukwudi and Yusha’u 2016). The phytochemicals detected in the extracts of *C. sativa* in the present study which have been detected in extract of *C. sativa* previously include flavonoids, sterols and alkaloids (Pollastro et al. 2018) and flavonoids, alkaloids, sterols, saponins, tannins and terpenoids in extracts of *Cannabis indica* (Pollastro et al. 2018). Cardiac glycosides and cardenolides, proteins and fatty acids were not detected in any of the solvent extractives. A study by Audu et al. (2014) using *C. sativa* L. procured from the National Drug Law Enforcement Agency (NDLEA) in Nigeria revealed a high presence of cardiac glycosides in petroleum ether (a non polar solvent) crude extract of C. sativa leaves. The absence of these biomolecules from the extracts of the solvents used in the present study to differences in the strain of *C. sativa* used and the differences in the climate, soil and topography between Lesotho (temperate climate with two-thirds of the terrain being mountainous and over 80% of soils in the lowlands being acidic) and Nigeria (tropics) where the plants were grown, and the different extraction methods used, petro-
leum ether (Audu et al. 2014) and methanol, hexane, chloroform and ethyl acetate in the present study (Ramelet 2015). The most ubiquitous classes were the polyphenols, anthocyanins, betacyanins, quinines, coumarins and amino acids which were detected in all the solvent extracts. These compounds were also detected in petroleum ether extracts of leaves of *C. sativa* in the study by Audu et al. (2014). The flavonoids, alkaloids, saponins, phlobatannins and anthraquinones were detected at different colour intensities only in the methanol extract while phytosterols were present at high intensity only in the hexane extract, since hexane is a non polar solvent. Terpenoids were detected only in the hexane and methanol extracts. Constituents such as carotenoids, terpenoids, ascorbates, reducing sugars and tocopherols are known to contribute to the antioxidant, antiviral, anticancer and anti-inflammatory properties of phenolic compounds (Bang et al. 2015; ElSohly et al. 2017; Andre et al. 2016). In another study by ElSohly et al. (2017) they identified the quinone 2-geranyl-5-hydroxy-3-n-pentyl-1,4-benzoquinone in extracts of leaves and buds of *C. sativa* using several chromatographic techniques. The differences in biological activities of different solvent fractions have been demonstrated by other researchers. Mihailović et al. (2013) studied the antioxidant and antigenotoxic activities of chloroform, ethyl acetate and n-butanol fractions obtained from the methanolic extract of *Gentiana asclepiadea* L. roots. Among all fractions, the ethyl acetate fraction exhibited the highest antioxidant activity, as well as total phenolics (146.64 GAE/g), flavonoids, flavonols and gallotannins contents.

Only the total phenolics content of crude hexane, chloroform, ethylacetate and methanol extracts of *C. sativa* were determined in this study. The determined value of total phenolics (Figure 1) of the hexane, chloroform, ethyl acetate and methanol extracts were, 39831.46, 2544.94, 2438.20 and 56601.12 mg GAE/gram dry weight respectively. The methanol extract, being the most polar, had the highest content of phenolics. In a study conducted by Mkpenie et al. (2012), the polyphenol content of the acetone and methanolic extracts of *C. sativa* was found to be in the range of 0.090 – 0.556 mg GAE/g dry weight. We therefore attribute the higher content of total phenolics observed in the present study to the different extraction times; 2, 8 and 18 hours in the study by Mkpenie et al. (2012) compared to the extraction time of 48 hours in the current study.

As shown in Tables 3 and 4, the concentrations of CP (1.25 mg mL⁻¹) and EMS (0.25 mg mL⁻¹) used in the present study did not reduce the mitotic index (MI) of meristem cells of the treated roots compared with the negative control, and were adjudged not cytotoxic. The concentrations of CP and EMS used however induced genotoxicity in the root meristem cells of *A. cepa*. In a study by Çelik and Aslantürk (2010), EMS at a concentration of 2x10⁻² M (0.2484 mg mL⁻¹) was both toxic and mutagenic to root meristem cells of *A. cepa*. CP at a concentration of 1% (1 mg mL⁻¹) was also both toxic and clastogenic to onion root meristem cells (Akeem et al. 2011).

The results of the assessments of the cytotoxic and genotoxic effects of the methanol and ethyl acetate extracts of *C. sativa* are presented in Tables 3 and 4. Only the lowest concentration (0.75 mg mL⁻¹) of the methanol extract and its mixture with EMS (0.25 mg mL⁻¹) (Table 3) and the lowest concentration (0.1875) of the ethyl acetate extract (Table 4), significantly reduced the (P+M)/(A+T) ratio. A decrease in the proportion of dividing cells in A+T is an indication of metaphase arrest due to the poisoning of the spindle fibers, akin to the action of the well documented spindle poison, colcemid (Parry et al. 1999). The chemotherapeutic agents taxol, vincristine, vinblastine and nocodazole act in a similar manner (Alberts et al. 2008). These compounds act by binding to and stabilizing microtubules, inhibiting their dynamic instability and causing various genetic disruption, including the induction of cell cycle arrest (Alberts et al. 2008; Zhang et al. 2015). In the present study, it seems that cell cycle arrest at the metaphase/anaphase junction by these extracts depended on concentration as only the lowest concentration (0.75 mg mL⁻¹) of the methanol extract and the lowest concentration (0.1875 mg mL⁻¹) of the ethylacetate extract induced mitotic cell arrest.

All three concentrations (0.75, 1.5 and 3.0 mg mL⁻¹) of the methanol extract of *C. sativa* and their individual mixtures with CP or EMS (Table 3) tested were cytotoxic to the onion root meristem cells. None of the three concentrations (0.1875, 0.375 and 0.75 mg mL⁻¹) of the ethylacetate extract of *C. sativa* alone or in mixtures with CP or EMS (Table 4) was cytotoxic to the onion root meristem cells. Plant secondary metabolites, such as the ones detected in the extracts of *C. sativa* in the present study, are the key drivers of the pharmacological actions of medicinal plants and have been shown to possess various biological effects including antibiotic, antifungal, antiviral and cytotoxic effects and therefore are able to protect plants from pathogens (Asche 2005; Hussein and El-Anssary 2018, Priyanka et al. 2019). The toxicity of the extracts observed in the present study was therefore attributed to the presence of cytotoxic secondary plant metabolites in the solvent extracts. In the Ames assay with extracts of *C. sativa* diluted with olive oil as well as the extracts produced with an isopropanol
Evaluation of extracts of wild Cannabis sativa L. for genotoxicity and phytochemical composition

and supercritical CO₂ extraction method, toxicity was evident for strains TA 98, TA 1535, TA 1537 and E. coli WP2 uvrA at ≥50 μg/plate, with and without S9, in the plate incorporation and/or pre-incubation tests (Dziwenka et al. 2020). These results are similar to results of other researches that demonstrated cytotoxicity of plant extracts including betel and tobacco leaf extracts and some Nigerian folk medicines to root-tip cells of A. cepa (Sopova et al. 1983; Abraham and Cherian 1978).

Regarding the genotoxicity of the extracts, all three concentrations (0.75, 1.5 and 3.0 mg mL⁻¹) of the methanol extract of C. sativa and their individual mixtures with CP or EMS (Table 3) tested were genotoxic to the onion root meristem cells. The 0.1875 and 0.375 mg mL⁻¹ dilutions of the ethyl acetate extract and their individual mixtures with CP and the mixture of 0.75 mg/mL ethyl acetate extract with CP were also genotoxic. In addition, the mixture of each concentration (0.375 or 0.75 mgmL⁻¹) of the ethyl acetate extract with EMS was also genotoxic.

The chromosomal abnormalities observed following treatment of the root tip cells of A. cepa with methanol and ethyl acetate extracts of C. sativa alone or in mixture with CP or EMS included sticky chromosomes, c-mitosis, chromosome laggards, Chromosome fragments, and anaphase and telophase bridges.

Genotoxic effects of different medicinal herbs in A. cepa have been demonstrated (Soliman 2001; Bidau et al. 2004; Çelik and Aslantürk 2007; Akinboro and Bakare 2007; Akintonwa et al. 2009; Oyedare et al. 2009). Marijuana smoke condensates were mutagenic to the TA98 strain in the Ames Salmonella/microsome bioassay but only in the presence of liver homogenates (Busch et al. 1979). However a supercritical CO₂ extract of the aerial parts of the C. sativa, was not mutagenic in the Ames bacterial reverse mutation test, in vitro mammalian chromosomal aberration test, or in an in vivo mouse micronucleus study (Marx et al. 2018). In another assessment of extracts of hemp (C. sativa) using the Ames reverse mutation assay, the extracts produced with an isopropanol and supercritical CO₂ extraction methods were diluted with olive oil and the undiluted extract formulated as a solution in DMSO; no mutagenic effect was observed in the four strains of Salmonella typhimurium (TA98, TA100, TA1535 and TA1537) and one strain of E. coli (WP2 uvrA) that were used (Dziwenka et al. 2020). In the present study, methanol and ethyl acetate extracts of the areal parts of C. sativa dissolved in 2.5% acetone as solvent, induced genotoxicity in the A. cepa root meristem cells.

The modulatory effects (ME) of the extracts on mutagen-induced genotoxicity are presented in columns 11 and 12 of Tables 3 and 4 for the methanol and ethyl acetate extract respectively. The ME indicated the type of interaction between the extract and the mutagen. The mixture of each concentration (0.75, 1.5 and 3.0 mg mL⁻¹) of the methanol extract with CP, Table 3, was significantly (>two-fold) more genotoxic than the mutagen (CP) alone with +ME values of (11.27, 11.89 and 6.43) respectively. The mixtures were also more genotoxic than the C. sativa extract alone at each concentration. The mixtures of the different concentrations (0.75, 1.5 and 3.0 mg mL⁻¹) of the methanol extract of C. sativa with EMS were also more genotoxic than the mutagen (EMS) alone with positive ME (+ME) values of (8.10, 1.61 and 2.14) respectively. Only the +ME values of the mixture of EMS with the lowest (0.75 mgmL⁻¹) or highest (3.0 mgmL⁻¹) concentration were significant, i.e. >two-fold the genotoxicity induced by the mutagen alone. These results indicated a synergistic interaction between each of the three concentrations of the methanol extract of C. sativa with CP and between two concentrations of the methanol extract C. sativa with EMS. The mixture of 0.1875 mgmL⁻¹ of the ethylacetate extract of C. sativa and CP (Table 4) was significantly more genotoxic (ME = 0.47) than the mutagen (CP) alone; the mixture of 0.375 or 0.75 mgmL⁻¹ of the ethylacetate extract with CP was insignificantly less genotoxic (ME = -0.50 and -0.73 respectively) than CP alone and therefore no interaction between CP and ethylacetate extract was inferred. Each mixture of 0.1875 or 0.375 mgmL⁻¹ of the ethylacetate extract with EMS was insignificantly less genotoxic than EMS alone with negative ME (-ME) values of -0.81 and 0.64 respectively. The mixtures were also less genotoxic than the ethylacetate extract alone. The mixture of 0.75 mgmL⁻¹ of the ethyl acetate extract with EMS was significantly more genotoxic (+ME value of 2.20) than EMS or C. sativa extract alone. The significant +ME value indicated a synergistic interaction between the ethyl acetate extract of C. sativa with EMS at the highest concentration only. These results demonstrated that the methanol and ethylacetate extracts of C. sativa did not exert any anti-genotoxic effects on CP- or EMS- induced genotoxicity. Lack of or differential anti-genotoxic activity of different solvent extracts have been demonstrated in other test systems. Mihailović et al. (2013) studied the antioxidant and antigenotoxic activities of chloroform, ethyl acetate and n-butanol fractions obtained from the methanolic extract of Gentiana asclepiadea L. roots and found no significant difference in the antigenotoxic effect of the different fractions against EMS-induced DNA damage in the in vivo sex linked recessive lethal mutations assay in Drosophila melanogaster males (Mihailović et al., 2013). The differential effects of different concentrations of
plant extracts or plant derivatives on mutagens-induced genotoxicity have been demonstrated in many test systems. In mice, an increase in the anticalcogenic activity of CP-induced clastogenicity by β-carotene at lower doses and an absence of a protective effect at higher concentrations were observed (Salvadori et al. 1992). Salvadori et al. (1992) interpreted the observations to mean different mechanisms of β-carotene modulation and a possible alteration of the balance of CP activation/detoxification mechanism of the promutagen. While no study on the antimutagenic activity of C. sativa extract was found, however, two pure terpenes that are found in cannabis (Bedini et al. 2016), D-linalool (LNL) and myrcene (MYR), were efficient against t-butyl hydroperoxide (t-BOOH) induced genotoxicity in the reverse mutation assay with E. coli and oxyR mutants and in the comet assay using cultured human hepatoma HepG2 and human B lymphoid NC-NC cells, which was predominately mediated by direct radical scavenging activity (Mitić-Čulafić et al. 2009). Another pure terpene, found in cannabis, bisabolol (BISA), caused a reduction in the levels of Aβ-induced chromosomal damage and apoptosis in PC12 cells (Shanmuganathan et al. 2018). It is now well accepted that the health benefits of fruits, vegetables and other plant foods are due to the synergy or interactions between the different bioactive compounds or other nutrients present in the whole foods, and not to the action of a sole compound (Liu, 2013). Similarly, we attribute the differences in the actions between the ethyl acetate and methanol extracts in the induction of cytotoxicity, genotoxicity and modulatory effects at different extract concentrations observed in this study to the synergistic or antagonistic interactions between various phytochemicals present in the extracts. According to Efferth and Koch (2011) cannabis-based therapeutics in humans, exert their pharmacological effects via synergistic or antagonistic interactions between the various phytochemicals it contains.

5. CONCLUSION

The order of effectiveness at extracting, from the aerial parts of A. sativa, the 19 different phytochemicals investigated in the present study was 95% methanol (15/19), followed by hexane and chloroform (9/19 each) and ethylacetate (7/19). Total phenolics content, in mg GAE/gram dry weight of extract was 95% methanol (5660.12) > ethylacetate (2438.20) > chloroform (2544.94) > hexane (3983.46). The methanol extract was both cytotoxic and genotoxic to the A. cepa root meristem cells, but the ethyl acetate extract was not cytotoxic but genotoxic. Mixtures of methanol extract (0.75, 1.5 and 3.0 mg mL⁻¹) with either CP or EMS were more genotoxic than the CP, EMS or extract alone which demonstrated a synergistic interaction between the methanol extract of C. sativa with CP and between two concentrations of the methanol extract with EMS. Mixtures of ethyl acetate extract of C. sativa (0.1875, 0.375, 0.75 mg/mL) with either CP or EMS were generally insignificantly (p<0.05) less genotoxic than CP, EMS or extract alone. Thus no interaction was observed between all three concentrations and the two lower concentrations of ethylacetate extract with CP or EMS. There was however a synergistic interaction between the highest concentration of the ethylacetate extract with EMS (+ ME of 2.2). The methanol and ethylacetate extracts of C. sativa did not exert any anti-genotoxic effects on CP- or EMS- induced genotoxicity.

The differences in the cytotoxicity and MEs of the extracts on the mutagens-induced genotoxicity were attributed to differences in phytochemical composition of the extracts.

REFERENCES

Abraham S, Cherian V D. 1978. Studies on cellular damage by extracts of betel leaves used for chewing. Cytologia. 43(1):203–228.
Akeem A, Mohamed KB, Asmawi MZ, Sofiman OA. 2011. Mutagenic and antimutagenic potentials of fruit juices of five medicinal plants in Allium cepa L.: Possible influence of DPPH free radical scavengers. African Journal of Biotechnology. 10(51):10520-10529.
Akinboro A, Bakare AA. 2007. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on Allium cepa Linn. Journal of Ethpharmacology. 112(3):470–475.
Akintonwa A, Awodele O, Afolayan G, Coker HA. 2009. Mutagenic screening of some commonly used medicinal plants in Nigeria. Journal of Ethpharmacology. 125(3): 461-470.
Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. 2008. Molecular Biology of the Cell, 1601, Garland Science, New York.
Andre CM, Hausman J-F, Guerriero G. 2016. Cannabis sativa: the plant of the thousand and one molecule. Frontiers in Plant Science. 7:19-26.
Asche C. 2005. Antitumour quinones. Mini Reviews in Medicinal Chemistry. 5(5): 449-467.
Ashton CH. 2001. Pharmacology and effects of cannabis: a brief review. The British Journal of Psychiatry. 178:101-106.
Asita AO, Moramang S, Rantsò T, Magama S. 2017. Modulation of mutagen-induced genotoxicity by vitamin C and medicinal plants in *Allium cepa*, L. Caryologia. 70(2): 151-165.

Audu BS, Ofokewwu PC, Ujah A, Ajima MNO. 2014. Phytochemical, proximate composition, amino acid profile and characterization of Marijuana (*Cannabis sativa* L.). The Journal of Phytopharmacology. 3(1):35-43.

Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. 2015. Phytochemistry of *Humulus lupulus* essential oils as novel control tools against the invasive mosquito *Aedes albopictus* and fresh water snail *Physella acuta*. Industrial Crops and Products. 85:318-323.

Bidai CJ, Amat AG, Yajia M, Marti DA, Riglos AG, Silvestroni A. 2004. Evaluation of the genotoxicity of aqueous extracts of *Ilex paraguariensis* St. Hil. (Aquifoliaceae) using the *Allium cepa* test. Cytologia. 69(2):109-117.

Bloomer J. 2019. Turning Cannabis Into Cash: Agrarian Change and Lesotho’s Evolving Experience. EchoGéo. 48(48). URL: http://journals.openedition.org/echogeo/17612

Bonciu E, Firbas P, Fontanetti CS, Wusheng J, Bloomer J. 2019. Turning Cannabis Into Cash: Agrarian Change and Lesotho’s Evolving Experience. EchoGéo. 48(48). URL: http://journals.openedition.org/echogeo/17612

Çelik TA, Aslantürk S (2010). Evaluation of cytotoxicity and genotoxicity of *Inula viscosa* leaf extracts with *Allium* Test. BioMed Research International. 2010(1):1-9.

Çelik TA, Aslantürk S. 2007. Cytotoxic and genotoxic effects of *Lavandula stoechas* aqueous extracts. *Phytochemistry of Cannabis sativa*. 62(3): 292-296.

Chukwudi IE, Yusha'u M. 2016. Phytochemical screening and brine shrimp lethality assay of the leaf extracts of *Cucurbita maxima*, *Euphorbia hirta*, *Leptadenia hastata* and *Mitracarpus scaber*. International Journal of Current Research in Life Sciences. 5(5):579-583.

Debisi S, Archana S, Geeta T. 1996. Plant extracts as modulators of genotoxic effects. The Botanical Review. 62(4):275-300.

Dziwenka M, Coppock R, McCorkle A, Palumbo E, Ramirez C, Lerner S. 2020. Safety assessment of a hemp extract using genotoxicity and oral repeat-dose toxicity studies in sprague-dawley rats. Toxicology Reports. 7: 376-385.

Effert H, Koch E. 2011. Complex interactions between phytochemicals. The multi-target therapeutic concept of phyotherapy. Current Drug Targets. 12(1):122-132.

Eid SY, El-Readi MZ, Wink M. 2012. Synergism of three-drug combinations of sanguinarine and other plant secondary metabolites with digitonin and doxorubicin in multi-drug resistant cancer cells. Phytomedicine. 19(14):1288-1297.

El-Alfy AT, Ivey K, Robinson K, Ahmed S, Radwan M, Slade D, Khan I, ElSohly M, Ross S. 2010. Antidepressant-like effect of Δ9-tetrahydrocannabinol and other cannabinoids isolated from *Cannabis sativa* L. Pharmacology Biochemistry and Behavior. 95 (4): 434–442.

ElSohly M, Gul W. 2014 In: Roger Pertwee, Editor. Handbook of Cannabis. Published to Oxford Scholarship Online: January 2015. Available from: https://www.oxfordscholarship.com/view/10.1093/acprof:oso/9780199662685.001.0001/acprof-9780199662685-chapter-1?print=pdf

ElSohly MA, Radwan MM, Gul W, Chandra S, Galal A. 2017. Phytochemistry of *Cannabis sativa* L. Progress in the Chemistry of Organic Natural Products. 103:1-36.

ElSohly MA, Slade D. 2005. Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life Sciences*. 78(5):539-48.

Fachinneto JM, Bagatini MD, Silva ACF, Tedesco SB. 2007. Efeito anti-proliferativo das infusões de *Achyrocline satureioides* DC (Asteraceae) sobre o ciclo celu-
lar de Allium cepa. Revista Brasileira de Farmacognosia 17(1):49-54.

Fachinetto JM, Tedesco SB. 2009. Atividade antiproliferativa e mutagênica dos extratos aquosos de Baccharis trimera (Less.) A. P. de Candolle e Baccharis articulata (Lam.) Pers. (Asteraceae) sobre o sistema teste de Allium cepa. Revista Brasileira de Plantas Medicinais 11(4):360-367.

Fayera S, Babu NG, Dekebo A, Bogale Y. 2018. Phytochemical Investigation and Antimicrobial Study of Leaf Extract of Plantago lanceolata. Natural Products Chemistry & Research. 6(2): 1-8.

Hales BE. 1982. Comparison of the mutagenicity and teratogenicity of cyclophosphamide and its active metabolites, 4 hydroxycyclophosphamide, phosphoramide mustard and acrolein. Cancer Research. 42(8):3016-3021.

Harborne AJ. 1998. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. Dordrecht: Springer: 1-302.

Herrera-Ruiz M, Roman-Ramos R, Zamilpa A, Tortorello J, Jimenez-Ferrer JE. 2008. Flavonoids from Tilia americana with anxiolytic activity in plus-maze test. Journal of Ethnopharmacology. 118(2):312-317.

Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. Lancet. 342(8878):1007-1011.

Higashimoto M, Purintrapiban J, Kataoka K, Kinouchi T, Vinikhetkumnuen U, Akimoto S, Matsumoto H, Ohnishi Y. 1993. Mutagenicity and antimutagenicity of extracts of three spices and a medicinal plant in Thailand. Mutation Research. 303(3):135–142.

Hussein RA, El-Ansary AA. 2018. Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants, Herbal Medicine, Philip F. Builders, IntechOpen. Available from: https://www.intechopen.com/books/herbal-medicine/plants-secondary-metabolites-the-key-drivers-of-the-pharmacological-actions-of-medicinal-plants.

Kassie F, Parzefall W, Musk S, Johnson I, Lamprecht G, Sontag G, Knasmuller S. 1996. Genotoxic effects of crude juices from Brassica vegetables and juices and extracts from phytopharmaceutical preparations and spices of cruciferous plants origin in bacterial and mammalian cells. Chemico-Biological Interactions. 102(1):1-16.

Khaidakov M, Bishop ME, Manjanatha MG, Lyn-Cook LE, Desai VG, Chen JJ, Aidoo A. 2001. Influence of dietary antioxidants on the mutagenicity of 7, 12-dimethylbenz[a]anthracene and bleomycin in female rats. Mutation Research. 480-481(1): 163-170.

Knoll MF, Silva ACF, Tedesco SB, Canto-Doros TS. 2006. Effects of Pterocaunon polyspathyllum DC. (Asteraceae) on onion (Allium cepa) root-tip cells. Genetics and Molecular Biology. 29(3):539-542.

Laniel L. 1999. Cannabis in Lesotho: a preliminary survey. "Discussion Paper n°34", Management of Social Transformations connected to the international drug problem (MOST-Drugs), Unesco, Paris. Available from: http://laniel.free.fr/INDEXES/PapersIndex/Cannabis_in_Lesotho_MOST.htm

Leme MD, Marin-Morales MA. 2009. Allium cepa test in environmental monitoring: a review on its application. Mutation Research. 682 (1): 71-81.

Liu RH. 2013. Health-promoting components of fruits and vegetables in the diet. Advances in Nutrition. 4(3):384S-392S.

Lu Y, Knoo T, Wiart C. 2014. Phytochemical analysis and antioxidant activity determination on crude extracts of Melodinus eugeniifolius barks and leaves from Malaysia. Pharmacology & Pharmacy. 5(8):773-780.

Lubini G, Fachinetto J, Laughinghouse H, Paranos J, Silva A, Tedesco S. 2008. Extracts affecting mitotic division in root-tip meristematic cells. Biologia. 63: 647-651.

Magama S, Rants’o T, Asita AO, Taole M. 2018. Evaluation of Procavia capensis hyraceum used in traditional medicine for antioxidant activity. Journal of Medicinal Plants Research. 12(19):248-257.

Marselos M, Karamanakos P. 1999. Mutagenicity, developmental toxicity and carcinogenicity of cannabis. Addiction Biology. 4(1): 5-12.

Marx TK, Reddeman R, Clewell AE, Endres JR, Béres E, Vértesi A, Glávits R, Hirka G, Szakonyiné IP. 2018. An assessment of the genotoxicity and subchronic toxicity of a supercritical fluid extract of the aerial parts of hemp. Journal of Toxicology. 2018:1-27.

McDonald S, Prenzler PD, Autolovich M, Robards K. 2001. Phenolic content and antioxidant activity of olive extracts. Food Chemistry. 73(1):73-84.

Mihailović V, Matić S, Mišić D, Solujić S, Stanić S, Katančić J, Mladenović M, Stanković N. 2013. Chemical composition, antioxidant and antigenotoxic activities of different fractions of Gentiana asclepiadea L. roots extract. Experimental and Clinical Sciences Journal. 12:807-823.

Mitić-Ćulafić D, Žegura B, Nikolić B, Vuković-Gačić M,Builders, IntechOpen. Available from: http://www.intechopen.com/books/herbal-medicine/plants-secondary-metabolites-the-key-drivers-of-the-pharmacological-actions-of-medicinal-plants.

Mkpenie VN, Essien EE, Udoh II. 2012. Effect of extraction conditions on total polyphenol contents, antioxi-
dant and antimicrobial activities of Cannabis sativa L. Electronic Journal of Environmental, Agricultural and Food Chemistry. 11(4): 300 – 307.

Mohn GR, Ellenberger J. 1976. Genetic effects of cyclophosphamid, ifosfamide and trofosfamide. Mutat Res. 32(3-4):331-360.

Moyo M, Amoo SO, Ncube B, Ndhlala AR, Finnie JF, Van Staden J. 2013. Phytochemical and antioxidant properties of unconventional leafy vegetables consumed in southern Africa. South African Journal of Botany. 84:65-71.

Mulyantingsih S, Sporer F, Zimmermann S, Reichling J, Wink M. 2010. Synergistic properties of the terpenoids aromadendrene and 1, 8-cineole from the essential oil of Eucalyptus globulus against antibiotic-susceptible and antibiotic-resistant pathogens. Phytomedicine. 17(13):1061-1066.

National Institute on Drug Abuse (NIDA). 2018. Marijuana as Medicine. Available from:https://www.drugabuse.gov/publications/drugfacts/marijuana-medicine.

Nwaoguikpe RN, Ujowundu CO, Emejulu AA. 2014. The antioxidant and free radical scavenging effects of extracts of seeds of some neglected legumes of south-east Nigeria. Scholars Academic Journal of Biosciences. 2(1): 51-59.

Oyedare BM, Bakare AA, Akinboro A. 2009. Genotoxicity assessment of water extracts of Ocimum gratissimum, Morinda lucida and Citrus medica using the Allium cepa assay. Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas. 8(2):97-103.

Padhi L, Panda SK. 2015. Antibacterial activity of Eleutherine bulbosa against multidrug-resistant bacteria. Journal of Acute Medicine. 5(3): 53-61.

Padmanabhan P, Sujana KA. 2008. Animal products in traditional medicine from Attappady hills of Western Ghats. Indian Journal of Traditional Knowledge. 7(2):326-329.

Parry EM, Mumba LE, Asita A, Parry JM. 1999. Mechanisms of action of aneuploidy inducing chemicals with particular reference to spindle inhibitors and neurotoxins. In: Trends in Environmental Mutagenesis. Sobti RC, Obe G, Quillardet P (Eds.), Tausco Book distributors, New Delhi, India, pp.101-110.

Pollastro F, Minassi A, Fresu L. 2018. Cannabis phenolics and their bioactivities. Current Medicinal Chemistry. 25(10):1160-1185.

Priyanka, Mohanka R, Kumari P, Kumar B. 2019. Cytotoxicity evaluation of aqueous extracts of medicinal plants on Allium cepa L. IOSR Journal of Biotechnology and Biochemistry. 5(2):35-49.

Ramelet A-A. 2015. Chap. 14. Veno-active drugs. In Goldman MP, Guex JJ, Weiss RA. Sclerotherapy, 5th ed. Saunders-Elsevier. Available from: https://clinicalgate.com/venoactive-drugs/

Ranotsi A, Makatjane T, Aiyuk S. 2012. The extent of drug abuse in Lesotho: The case of Mapoteng community. Lesotho Medical Association Journal. 10(1):11-18.

Razak MR, Afzan A, Ali R, Jajaluddin A, Wasiman M, Zahari S, Abdullah N, Ismail Z. 2014. Effect of selected local medicinal plants on the asexual blood stage of chloroquine resistant Plasmodium falciparum. BMC Complimentary and Alternative Medicine 14:492.

Salvadori DMF, Ribeiro LR, Oliveira MDM, Pereira CAB, Beçak W. 1992. The protective effect of β-carotene on genotoxicity induced by cyclophosphamide. Mutation Research. 265(2):237-244.

Schimmer O, Kruger A, Paulini H, Haefele F. 1994. An evaluation of 55 commercial plant extracts in the Ames mutagenicity test. Pharmazie. 49(6):448–451.

Shale TL, Stirk WA, Van Staden J. 1999. Screening of medicinal plants used in Lesotho for anti-bacterial and anti-inflammatory activity. Journal of Ethnopharmacology. 67(3): 347–354.

Shanmuganathan B, Suryanarayanan V, Sathya S, Narenkumar M, Singh SK, Ruckmani K, Devi KP. 2018. Anti-amyloidogenic and antiapoptotic effect of α-bisabolol against Aβ induced neurotoxicity in PC12 cells. European Journalof Medicinal Chemistry. 143:1196-1207.

Siegel RK. 1977. Cocaine: recreational use and intoxication. Pp. 119-136 in R.C. Petersen and R.C. Stillman, eds., Cocaine: 1977. NIDA Research Monograph 13. DHHS Pub. No. (ADM) 77-741. Rockville, Md.: National Institute on Drug Abuse.

Soliman MI. 2001. Genotoxicity testing of neem plant (Azadirachta indica A. Juss.) using the Allium cepa chromosome aberration assay. Journal of Biological Sciences. 1(11):1021-1027.

SonI A, Sheetal S. 2013. Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. Journal of Pharmacognosy and Phytochemistry. 2(4): 22-29.

Sopova M, Sekovski Z, Jovanovska M. 1983. Cytological effects of tobacco leaf extract on root-tip cells of Allium sativum. Acta Biologiae et Medicinae Experimentalis.8:49-56.

Stubbs L, Carver EA, Cachetei NL, Shelby M, Generoso W. 1997. Generation and characterization of heritable reciprocal translocations in mice. Methods. 13(4): 397-408.
tava J, editor. Environmental contamination. Croatia: InTech: p. 137–156.

Trease GE, Evans CW. 1984. Pharmacognosy, 12th ed. Bariere Tindall, London, UK, London.

Trease GE, Evans WC. 2002. Textbook of Pharmacognosy. 15th Ed. Saunders Publishers, London.

Turner CE, ElSohly MA, Boeren EG. 1980. Constituents of Cannabis sativa L. XVII. A review of the natural constituents. Journal of Natural Products. 43(2):169–234.

Uddin G, Feroz S, Ali J, Rauf A. 2014. Antioxidant, antimicrobial activity and phytochemical investigation of Pterospermum acerifolium (Leaf petiole). Wudpecker Journal Agriculture Resource. 3(3): 058-062.

van Wyk B-E, Wink M. 2015. Phytomedicines, Herbal Drugs and Poisons. Briza, Kew Publishing, Cambridge University Press: Cambridge, UK.

Wink M. 2015. Modes of Action of Herbal Medicines and Plant Secondary Metabolites. Medicines, 2(3):251-286

Wong FC, Chai TT, Hoo YW (2012). Antioxidation and cytotoxic activities of selected medicinal herbs used in Malaysia. Journal of Medicinal Plants Research. 6(16): 3169-3175.

Zhang CZ, Spektor A, Cornils H, Francis JM, Jackson EK, Liu S, Meyerson M, Pellman D. 2015. Chromothripsis from DNA damage in micronuclei. Nature. 522:179–184.

Zhang Z, Chang Q, Zhu M, Huang Y, Ho WKK, Chen ZY. 2001. Characterization of antioxidants present in hawthorn fruits. The Journal of Nutritional Biochemistry. 12(3): 144-152.

Zimmerman S, Zimmerman A. 1990. Genetic effects of marijuana. International Journal of Addictions. 25(1): 19-33.