http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2020.995.1003

Research Article
Cytotoxicity and Chromatographic Fingerprinting of *Euphorbia* Species Used in Traditional Medicine

[1]Setlabane Tebogo Michael Mampa, [1]Samson Sitheni Mashele and [2]Mamello Patience Sekhoacha

[1]Department of Health and Environmental Sciences, Central University of Technology, 9300 Bloemfontein, Republic of South Africa
[2]Department of Pharmacology, University of the Free State, P.O. Box 339, 9300 Bloemfontein, Republic of South Africa

Abstract
Background and Objective: Chromatographic fingerprinting of plant species play an important role in species identification and standardization of plant based health products. Some of the *Euphorbia* species are used in folk medicine, yet majority of these exhibit various degrees of toxicity. It becomes a challenge to distinguish the toxic from the non-toxic species. The study aimed to evaluate cytotoxicity and to determine the method for fingerprinting the chemical constituents of the selected *Euphorbia* species to identify markers of toxicity. **Materials and Methods:** Hexane, DCM, ethyl acetate and methanol extracts of *E. arabica*, *E. bupleurifolia*, *E. enopla*, *E. gorgonis, E. horrida* indigenous and *E. horrida* var. were examined in mammalian vero cell line using MTT cell viability test assay. The presence of secondary metabolites and proteins were assessed in the plant extracts and thin layer chromatography was used to identify toxicity markers. **Results:** The hexane and DCM extracts of *E. arabica*, *E. bupleurifolia* and the DCM extract of *E. horrida* var. exhibited the highest cell growth inhibition reaching IC_{50} at a concentration of 10 µg mL^{-1}. Both polar and non-polar extracts of *E. enopla* exhibited cell growth inhibition with the hexane extract reaching IC_{50} at a concentration of 10 µg mL^{-1}. *Euphorbia gorgonis* and *E. horrida* indigenous were not active against the vero cell line. Secondary metabolites were detected, however, proteins were not detected in all six *Euphorbia* species. The TLC profiles of toxic extracts revealed additional bands which were absent in non-toxic species. **Conclusion:** It is concluded that the TLC method developed in this study can be used as a quick screen method to possibly distinguish toxic from non-toxic species, as well as in identifying the studied species.

Key words: *Euphorbia*, phytochemical analysis, cytotoxicity, protein detection, vero cell line

Citation: Setlabane Tebogo Michael Mampa, Samson Sitheni Mashele and Mamello Patience Sekhoacha, 2020. Cytotoxicity and chromatographic fingerprinting of *Euphorbia* species used in traditional medicine. Pak. J. Biol. Sci., 23: 995-1003.

Corresponding Author: Mamello Patience Sekhoacha, Department of Pharmacology, University of the Free State, P.O. Box 339, 9300 Bloemfontein, Republic of South Africa

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Application of analytic chromatographic techniques for fingerprinting of plant species has brought solutions to challenges of species identification, qualitative and quantitative analysis of plants' constituents, as well as standardization of plant-based health products. Long before introduction of western medicine, plants were considered a valuable source of medicine. Medicinal plants are still preferred in their natural form as they are claimed to have less side effects compared to pharmaceutical drugs[1,2]. However, it remains a major problem to distinguish between similar species and to determine the desired constituents that may be present at the effective levels. Inadequate scientific information on standardization of plant extracts affects the efficacy of treatments and consistency of treatment outcomes. It is necessary to have a proper identification of plants through chemical fingerprinting in order to have a library that can be used to identify plant species for quality and quantity assessment[3].

Euphorbia is a highly diverse genus of flowering plants in the family *Euphorbiaceae*[4]. *Euphorbia* species have been reported to be toxic and this toxicity is mostly found in the white milky sap called latex, which has been reported to be harmful to humans and livestock[5-7]. Literature has reported that the latex and the aerial parts of *Euphorbia* species have historically been used to treat different ailments including cancer, wounds, warts and headaches[8-9]. Some species in this genus have some pharmacological properties such as; antiviral, anticancer, antimicrobial and anti-fungal properties[5].

The widespread use of some *Euphorbia* species in folk medicine necessitates that the toxic species and non-toxic species and/or medicinally useful species can be distinguished in order to categorize the species for their suitable applications.

Euphorbia species have also been reported to contain biologically active proteins such as proteases, chitinases, oxidases and lectins and have various phytochemicals such as, steroids, phenolic, cerebrosides, glycerols, flavonoids, glycosides, tannins, saponins, alkaloids, pentose, anthraquinones, phytosterols, terpenes including; diterpenes and triterpenes[10-13]. The presence of both phytochemical constituents and proteins implies different extraction and identification methods for these plant species. It is therefore necessary that different application methods are applied to obtain the ones suitable for fingerprinting the chemical constituents of these species to identify markers responsible for toxicity.

This study evaluated cytotoxic effects of *Euphorbia* species (*E. arabica*, *E. bupleurifolia*, *E. enopla*, *E. gorgonis*, *E. horrida* var and *E. horrida* indigenous) on vero cell line *in vitro*. The chemical fingerprinting will assist in quick screening of most *Euphorbia* species to determine whether the species is toxic or not and to help determine whether the tested species contains the necessary chemical composition for the intended application.

MATERIALS AND METHODS

Plant collection and extraction: The study was conducted at the Central University of Technology and University of the Free State in Bloemfontein, South Africa, from July, 2018-September, 2019. Six species of *Euphorbia* were collected from KwaZulu-Natal province of South Africa and Lesotho. Plants were authenticated by a botanist at University of the Free State. Fresh plants were chopped into small pieces, left to dry at room temperature and ground to fine powder. Crude extract was obtained by homogenizing 10 g of powdered material with 100 mL of organic solvents in their increasing order of polarity starting with hexane, dichloromethane, ethyl acetate and methanol.

Mixtures were left on a shaker for 48 h (FMH instruments, sepsci), then filtered with a filter paper (Whatman® Maidstone). Filtrates were dried by rotary evaporation (Buchi, labortechnik Switzerland) at 45°C, then placed under fume hood until dry. Dried extracts were stored at 4°C until further use. The percentage yields were calculated.

Phytochemical screening: The powdered plant materials of *E. arabica*, *E. bupleurifolia*, *E. enopla*, *E. gorgonis*, *E. horrida* var. and *E. horrida* indigenous were screened for phytosterols, pentose, tannins, glycosides, triterpenoids, anthraquinones, saponins, flavonoids and alkaloids based on the protocols[14,15].

Cytotoxicity screening: The mammalian vero cell line was obtained from cellonex, South Africa. Cells were cultured in complete medium; DMEM supplemented with 10% Fetal Bovine Serum (FBS) and maintained in an incubator (NUVE EC 160) at 37°C including 5% CO_2. Cells were sub-cultured at 90% confluency by trypsinization. Cells were centrifuged at 800 rpm for 5 min to obtain a cell pellet. Cells were re-suspended in 5 mL of the medium, viability of the cells was assessed using trypan crystal blue dye and cells were counted using automated cell counter (countess FL, life technologies). The concentration of the cells was calculated to obtain 1×10^5 cells mL^{-1} for plating in 96 well plates. Plates were incubated for 24 h at 37°C temperature. Following incubation, cells were treated with 100 μL of test extracts added in triplicates. The stock solutions of the test samples (20 mg mL^{-1}) were prepared in DMSO, diluted to concentrations of 100, 10 and 1 μg mL^{-1} in complete medium. Emetine was used as control standard drug. The plates were then incubated at 37°C for 48 h. Cell viability was measured using the MTT assay[16]. Results were analyzed using Microsoft excel.

Protein detection: Methanol extracts of the plants were dissolved and prepared in warm distilled water. The extracts were tested for presence of proteins using biuret and xanthoproteic tests. For Biuret test, Sodium Hydroxide (NaOH) and a few drops of Copper Sulfate ($CuSO_4$) solutions were added to the sample solution[17]. A violet or pink colour was observed. For xanthoproteic test, concentrated sulfuric acid (H_2SO_4) was added to the sample solution. A white precipitate was formed. In both tests, egg white was used as positive control.

Plant extract fingerprinting by TLC: Silica gel on thin aluminium plates (5×10 cm) was used as stationary phase. For mobile phase, three different solvent systems: Toluene-acetone (8:2) (non-polar solvent), Toluene-chloroform-acetone (40:25:35) (Semi-polar solvent) and n-butanol-glacial acetic acid-water (50:10:40) (Polar solvent) were used in elution[18]. Dried extracts were reconstituted (2 mg mL^{-1}) in the solvent used for extraction. The plates were developed in the appropriate mobile system. The TLC plates were visualised under ultraviolet (UV) light. The retention factors were calculated for every spot visible on the TLC plate. The R_f values were used to compare the chemical profiles of plant extracts to identify the presence/absence of toxicity markers in different plant species of *Euphorbia*.

Statistical analysis: The values are presented as the mean±standard deviation (SD).

RESULTS

The percentage yield of the dried plant extracts was calculated and results are summarised in Table 1. Generally, DCM and methanol had the highest yields in all plants extracted.

All six *Euphorbia* species confirmed the presence of phytosterols, glycosides, triterpenoids and flavonoids. Pentose was only found in *E. horrida* indigenous and *E. horrida* var. Saponins were detected in *E. bupleurifolia*, *E. horrida* indigenous and *E. horrida* var. Alkaloids were present in most species; *E. bupleurifolia*, *E. enopla*, *E. gorgonis* and *E. horrida* var. (Table 2).

Table 1: Yields (%) of six *Euphorbia* species following extraction with different solvents

	Yield (%) per solvent			
Plant samples	Hexane	DCM	MeOH	EtOAc
E. arabica (whole plant)	1.11	0.96	14.65	1.75
E. bupleurifolia (whole plant)	41.49	0.68	12.47	1.13
E. enopla (whole plant)	12.69	19.05	19.05	6.35
E. gorgonis (whole plant)	2.60	4.30	17.40	0.40
E. horrida indigenous (whole plant)	5.45	1.12	3.85	0.16
E. horrida var. (whole plant)	8.65	0.39	3.15	0.13

DCM: Dichloromethane, MeOH: Methanol, EtOAc: Ethyl acetate

Table 2: Phytochemical screening of *Euphorbia* species

	Euphorbia species					
Phytochemicals	*E. arabica*	*E. bupleurifolia*	*E. enopla*	*E. gorgonis*	*E. horrida* indigenous	*E. horrida* var.
Phytosterols	+	+	+	+	+	+
Pentose	-	-	-	-	+	+
Tannins	+	+	+	-	+	+
Glycosides	+	+	+	+	+	+
Triterpenoids	+	+	+	+	+	+
Anthraquinones	+	-	+	-	+	+
Saponins	-	+	-	-	+	+
Flavonoids	+	+	+	+	+	+
Alkaloids	-	+	+	+	-	+

+: Present, -: Absent

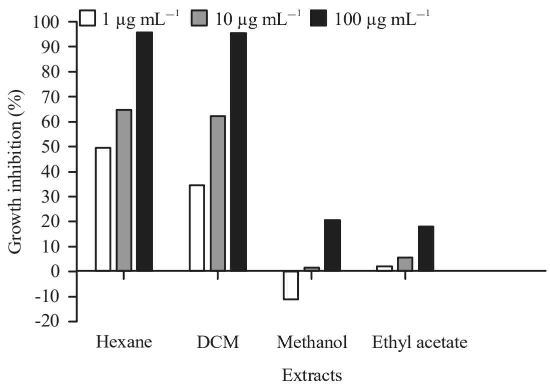


Fig. 1: Cell growth inhibitory effects of *E. arabica* extracts on vero cells

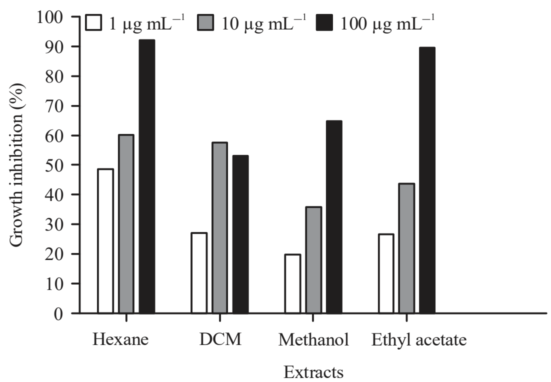


Fig. 2: Cell growth inhibitory effects of *E. bupleurifolia* extracts on vero cells

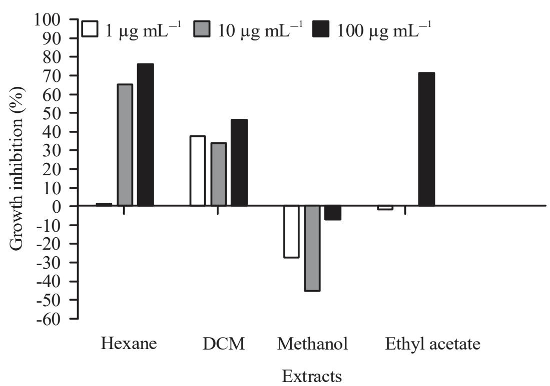


Fig. 3: Cell growth inhibitory effects of *E. enopla* extracts on vero cells

Cytotoxicity screening: The following graphs show cell growth inhibition effects of extracts of *E. arabica*, *E. bupleurifolia*, *E. enopla, E. gorgonis, E. horrida* indigenous and *E. horrida* var. Extracts that attained an IC_{50} at a concentration of 10 µg mL^{-1} and below were considered active.

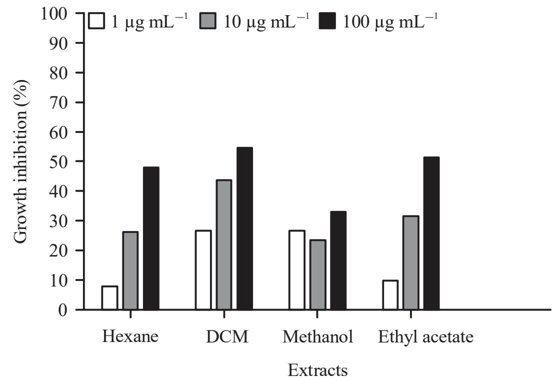


Fig. 4: Cell growth inhibitory effects of *E. gorgonis* extracts on vero cells

Hexane extracts of *E. arabica* exhibited the highest cell growth inhibition reaching IC_{50} at all concentrations tested, the DCM extract reached IC_{50} at 10 µg mL^{-1}. The methanol and ethyl acetate extracts of this *Euphorbia* didn't show any activity (Fig. 1).

Figure 2 shows that all four extracts of *E. bupleurifolia* showed varying cytotoxicity, with hexane and DCM extracts showing IC_{50} values at concentrations of 1 and 10 µg mL^{-1}, respectively.

Only the highly non-polar hexane extract of *E. enopla* exhibited considerable cell growth inhibition, at a concentration of 10 µg mL^{-1}. Interestingly, proliferation of cells was observed at concentrations of 1, 10 and 100 µg mL^{-1} for methanol extracts (Fig. 3).

The DCM and ethyl acetate extracts of *E. gorgonis* reached the IC_{50} value only at a concentration of 100 µg mL^{-1} (Fig. 4).

Hexane and DCM extracts of *E. horrida* indigenous reached IC_{50} only at a concentration of 100 µg mL^{-1}. Again, proliferation of vero cells was observed at a concentrations of 1, 10 and 100 µg mL^{-1} for methanol extracts (Fig. 5).

The DCM extract of *E. horrida* var. reached IC_{50} at a concentration of 10 µg mL^{-1}. Hexane and ethyl acetate extracts showed activity only at a concentration of 100 µg mL^{-1} (Fig. 6).

Protein detection: *Euphorbia* species have been reported to contain biologically active proteins. In this study, presence of proteins in the plant extracts was detected using biuret and xanthoproteic tests. For biuret test, a violet or pink colour was not observed as indicated in Table 3. For xanthoproteic test, a white precipitate was not formed as indicated in Table 3. Positive control results are shown in Fig. 7-8. Table 3 shows protein detection results for *E. arabica*, *E. bupleurifolia*, *E. enopla*, *E. gorgonis*, *E. horrida* indigenous and *E. horrida* var.

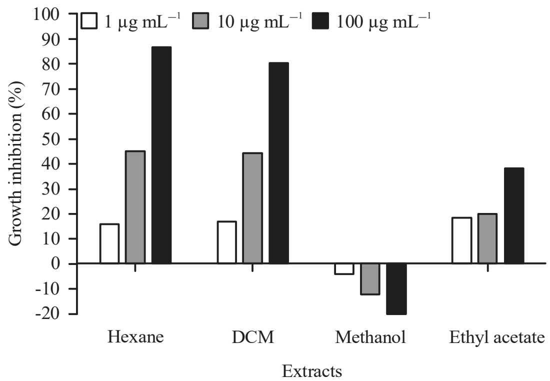


Fig. 5: Cell growth inhibitory effects of *E. horrida* indigenous extracts on vero cells

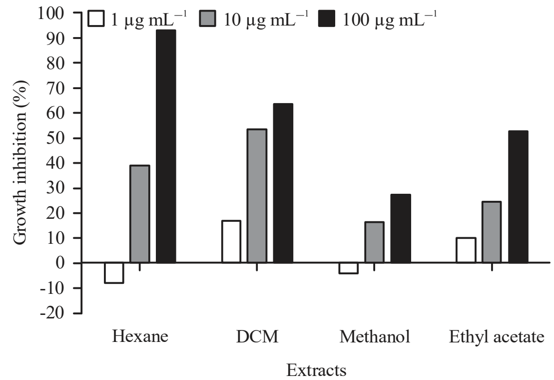
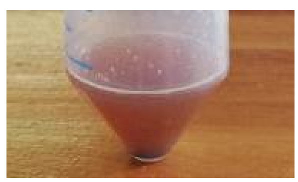
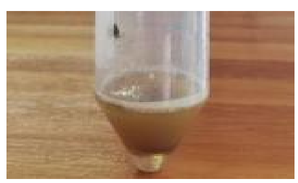
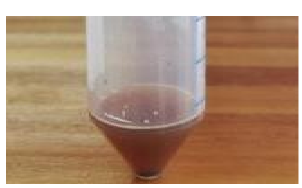
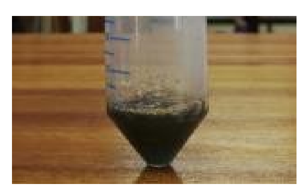


Fig. 6: Cell growth inhibitory effects of *E. horrida* var. extracts on vero cells

Table 3: Protein detection results of six *Euphorbia* species The whole plant was tested

Name of species	Xanthoproteic test	Biuret test
E. arabica		
E. bupleurifolia		
E. enopla		
E. gorgonis		
E. horrida indigenous		
E. horrida var.		

In all the plant species, proteins were not detected using both tests

Fig. 7: Biuret test using egg white

Fig. 8: Xanthoproteic test using egg white

Figure 9 shows the TLC profiles of all extracts. The toxic extracts, based on the cell culture results revealed additional bands which were absent in non-toxic species.

Thin layer chromatography: The TLC profiling results of hexane extracts showed that *E. bupleurifolia* had the highest number of bands followed by *E. enopla* and *E. horrida* var. with 6 bands; *E. arabica* and *E. horida* indigenous with 4 bands; *E. gorgonis* with no bands. The number of bands produced when visualized under UV light and the R_f values determined (Table 4).

The TLC profiling results of DCM extracts showed that *E. enopla* and *E. horrida* indigenous had the highest number of bands, followed by *E. bupleurifolia* and *E. horrida* var. with 11 bands each; *E. arabica* with 7 bands and *E. gorgonis* with no bands (Table 5).

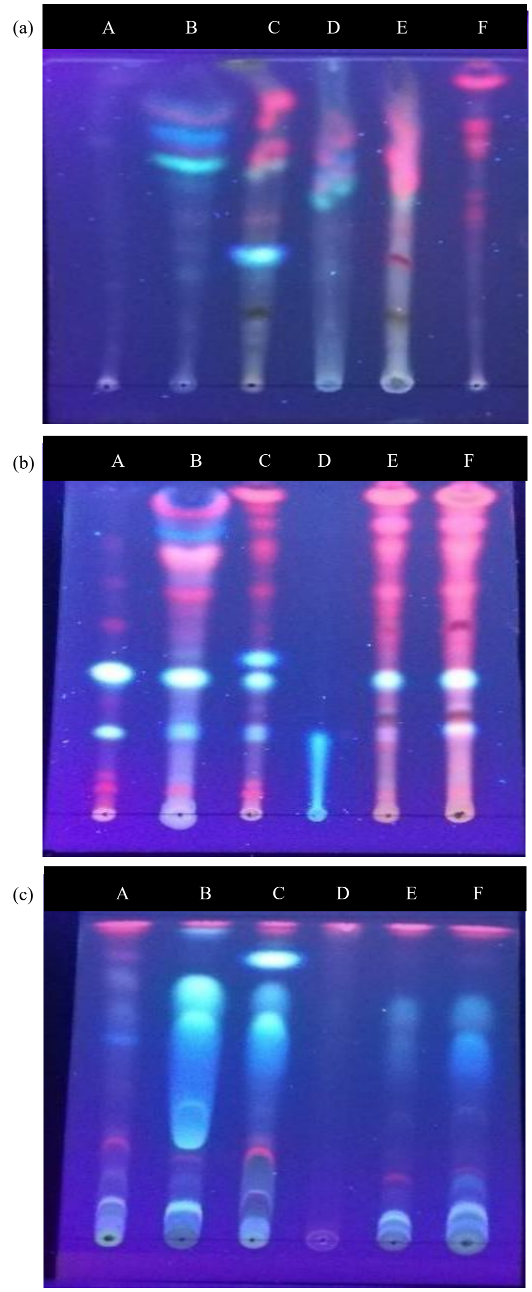


Fig. 9(a-c): TLC profiling results of (a) Hexane extracts, (b) DCM extracts and (c) Methanol extracts
Euphorbia species, A: *E. arabica*, B: *E. bupleurifolia*, C: *E. enopla*, D: *E. gorgonis*, E: *E. horrida* indigenous and F: *E. horrida* var. ethyl acetate and water extracts were not done due to inadequate plant material

TLC profiling results of methanol extracts showed that *E. arabica* had the highest number of bands followed by *E. enopla* with 8 band; *E. bupleurifolia*, *E. horrida* indigenous and *E. horrida* var. with 7 bands each and *E. gorgonis* with 1 band (Table 6).

Table 4: TLC profiling results of hexane extracts

Samples	Number of bands	R_f values
E. arabica	4	0.45, 0.82, 0.88, 0.90
E. bupleurifolia	8	0.4, 0.45, 0.48, 0.55, 0.77, 0.79, 0.82, 0.84
E. enopla	6	0.4, 0.48, 0.55, 0.79, 0.82, 0.84
E. gorgonis	0	0.00
E. horrida indigenous	4	0.4, 0.48, 0.55, 0.69
E. horrida var.	6	0.69, 0.71, 0.79, 0.82, 0.89, 0.91

Table 5: TLC profiling results of DCM extracts

Samples	Number of bands	R_f values
E. arabica	7	0.28, 0.31, 0.4, 0.48, 0.53, 0.59, 0.64
E. bupleurifolia	11	0.28, 0.31, 0.4, 0.47, 0.50, 0.51, 0.54, 0.59, 0.65, 0.71, 0.79
E. enopla	12	0.28, 0.31, 0.4, 0.47, 0.50, 0.51, 0.54, 0.59, 0.65, 0.71, 0.79, 0.82
E. gorgonis	0	0.00
E. horrida indigenous	12	0.28, 0.31, 0.4, 0.47, 0.50, 0.51, 0.54, 0.59, 0.65, 0.71, 0.79, 0.82
E. horrida var.	11	0.28, 0.4, 0.47, 0.50, 0.51, 0.54, 0.59, 0.65, 0.71, 0.79, 0.82

Table 6: TLC profiling results of methanol extracts

Samples	Number of bands	R_f values
E. arabica	9	0.48, 0.53, 0.59, 0.64, 0.69, 0.73, 0.87, 0.91, 0.94
E. bupleurifolia	7	0.48, 0.53, 0.59, 0.64, 0.69, 0.91, 0.94
E. enopla	8	0.48, 0.53, 0.59, 0.64, 0.69, 0.88, 0.91, 0.94
E. gorgonis	1	0.94
E. horrida indigenous	7	0.48, 0.53, 0.59, 0.64, 0.69, 0.72, 0.94
E. horrida var.	7	0.48, 0.53, 0.59, 0.64, 0.69, 0.72, 0.94

DISCUSSION

The study suggested classification of cytotoxic and non-cytotoxic species of *Euphorbia* based on cytotoxicity screening, phytochemical screening and profiling. Phytochemical analysis confirmed the presence of phytosterols that have been reported to have potential health benefits[19]. Pentose was only detected in *E. horrida* indigenous and *E. horrida* var. and this has been reported to reduce cytotoxicity of plant extracts[20]. This could be due to the sugar providing nutrients to the cells. This supported the low activity observed in these two species. Previous studies[21-24] reported that tannins cause regression of tumors that are already present in tissue, implying their potential in anti-proliferation of cancer cells activity. *Euphorbia gorgonis* did not show the presence of tannins in which could have added to the lack of anti-proliferation activity observed.

All six *Euphorbia* species showed the presence of triterpenoids, glycosides and flavonoids. These have been reported to exhibit innumerable biological and pharmacological activities such as antioxidant, anti-inflammatory and anti-cancer properties. These secondary metabolites have also implicated growth inhibition of cell lines through induction of apoptosis[25-28]. *E. arabica*, *E. enopla*, *E. horrida* indigenous and *E. horrida* var. showed the presence of anthraquinones. Literature has reported that anthraquinones detected in plant extracts are increasingly used for pharmaceuticals due to their therapeutic and pharmacological properties[29].

Toxicity is regarded as a secondary function of alkaloids[30], which support cytotoxicity exerted by *E. bupleurifolia*, *E. enopla* and *E. horrida* var. Although *Euphorbia* species are reported to contain biologically active proteins[13], in this study proteins were not detected in all six *Euphorbias*. The cytotoxicity of the plants could result primarily from the presence of secondary metabolites (phytochemicals). The results from this study suggested that the cytotoxic molecules in the studied *Euphorbia* plants are non-polar, since only the non-polar extracts showed activity while the more polar extracts were not active. Furthermore, the study focused on fingerprinting of phytochemical constituents of studied *Euphorbia* species, which can be used for identification of species for quality control purposes[31].

Species with the highest bands produced in the TLC profiles imply high amount of chemically varied phytochemicals. Based on the results obtained, *Euphorbia* extracts with less R_f values were considered more polar, which means that they stick to the stationary phase a lot stronger than *Euphorbia* extracts with more R_f values and therefore, moves slower in the mobile phase. Due to presence of various phytochemicals within extracts, it is difficult to attribute cytotoxicity effect to a specific phytochemical. However, further study is required to determine the exact toxicity markers responsible for activity. Active constituents could be isolated and further studied as antiviral, anticancer, antimicrobial and anti-fungal properties.

CONCLUSION

The *Euphorbia* species investigated in this study had a similar composition of phytochemicals, (phytosterols, glycosides, triterpenoids and flavonoids). Phytochemicals present in the species are known to possess various pharmacological activities, which support the use of *Euphorbia* species to treat various health conditions. The cytotoxicity exhibited by hexane and DCM extracts of *E. arabica*, *E. bupleurifolia*, *E. enopla* and *E. horrida* var. provide scientific preliminary evidence for their use in treatment of cancer. The clear differences in the TLC chemical profiles of the toxic and non-toxic species show the effectiveness and reliability of methods for application as a quick screening to either verify the species or determine the toxicity of the species.

SIGNIFICANCE STATEMENT

This study discovers the different levels of cytotoxicity of six *Euphorbia* species (*E. arabica*, *E. bupleurifolia*, *E. enopla*, *E. gorgonis*, *E. horrida* indigenous and *E. horrida* var.) that can be beneficial for fingerprinting of medicinal plants for use to distinguish and confirm the presence of secondary metabolites of interest. This study will help the researcher to uncover the critical areas of analytic chromatographic techniques used to screen species used for medicinal purposes, as the presence of chemical constituents such as secondary metabolites (phytochemicals) affect the efficacy and safety of the outcome of treatment.

ACKNOWLEDGMENTS

The authors express sincere thanks to Central University of Technology and University of the Free State, Bloemfontein, South Africa, for providing facilities to conduct these studies and National Research Foundation of South Africa for funding.

REFERENCES

1. Rios, J.L. and M.C. Recio, 2005. Medicinal plants and antimicrobial activity. J. Ethnopharmacol., 100: 80-84.
2. Kamboj, V.P., 2000. Herbal medicine. Curr. Sci., 78: 35-39.
3. Cos, P., A.J. Vlietinck, D.V. Berghe and L. Maes, 2006. Anti-infective potential of natural products: How to develop a stronger *in vitro* proof-of-concept. J. Ethnopharmacol., 19: 290-302.
4. Abou-El-Hamd, E.M., F.H. Mohamed-Elamir, M.F.M. Moustafa, M.A. El-Sayed and I.B. Abdel-Farid *et al*., 2012. *Euphorbia helioscopia*: Chemical constituents and biological activities. Int. J. Phytopharmacol., 3: 78-90.
5. Barla, A., H. Birman, S. Kültür and S. Öksüz, 2006. Secondary metabolites from *Euphorbia helioscopia* and their vasodepressor activity. Turk. J. Chem., 30: 325-332.
6. Chaudhry, B.A., K.H. Janbaz, M. Uzair and A.S. Ejaz, 2001. Biological studies of *Conyza* and *Euphorbia* species. J. Res. Sci., 12: 85-88.
7. Jassbi, A.R., 2006. Chemistry and biological activity of secondary metabolites in *Euphorbia* from Iran. Phytochemistry, 67: 1977-1984.
8. Krstić, G., B. Anđelković, Y.H. Choi, V. Vajs, T. Stević, V. Tešević and D. Gođevac, 2016. Metabolic changes in *Euphorbia palusrtis* latex after fungal infection. Phytochemistry, 131: 17-25.
9. Yang, Z.S., G.D. Chen, Y.X. Li and J. Chen, 2009. Characterization of callus formation in leaf of *Euphorbia helioscopia*. Afr. J. Plant Sci., 3: 122-126.
10. Sytwala, S., F. GŘnther and M.F. Melzig, 2015. Lysozyme-and chitinase activity in latex bearing plants of genus *Euphorbia*-A contribution to plant defense mechanism. Plant Physiol. Biochem., 95: 35-40.
11. Vogg, G., E. Mattes, J. Rothenburger, N. Hertkorn, S. Achatz and H. Sandermann Jr., 1999. Tumor promoting diterpenes from *Euphorbia leuconeura* L. Phytochemistry, 51: 289-295.
12. Villanueva, J., L.M. Quirós and S. Castañón, 2015. Purification and partial characterization of a ribosome-inactivating protein from the latex of *Euphorbia trigona* Miller with cytotoxic activity toward human cancer cell lines. Phytomedicine, 22: 689-695.
13. Carlini, C.R. and M.F. Grossi-de-Sa, 2002. Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. Toxicon, 40: 1515-1539.
14. Bhandary, S.K., S.N. Kumari, V.S. Bhat, K.P. Sharmila and M.P. Bekal, 2012. Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruit and seeds. J. Health Sci., 2: 34-38.
15. Yusuf, A.Z., A. Zakir, Z. Shemau, M. Abdullahi and S.A. Halima, 2014. Phytochemical analysis of the methanol leaves extract of *Paullinia pinnata* Linn. J. Pharmacogn. Phytother., 6: 10-16.
16. Direko, P., H. Mfengwana, S. Mashele and M. Sekhoacha, 2019. Investigating the angiogenic modulating properties of *Spirostachys africana* in MCF-7 breast cancer cell line. Int. J. Pharmacol., 15: 970-977.

17. Maurya, S.K., A. Asthana, S.P. Maurya, P. Maurya and A. Maurya, 2019. Qualitative analysis of protein: Egg albumin and milk. Indian J. Drugs, 7: 30-33.
18. Upadhyay, B., K.P. Singh and A. Kumar, 2010. Ethno-medicinal, phytochemical and antimicrobial studies of *Euphorbia tirucalli* L. J. Phytol., 2: 65-77.
19. Trautwein, E.A. and I. Demonty, 2007. Phytosterols: Natural compounds with established and emerging health benefits. Oleagineux Corps Gras Lipides, 14: 259-266.
20. Sake, K., R. Bugude, L.V. Reddy and P.S.S.V. Khan, 2013. Production of bioethanol from spent residues of latex yielding plants *Euphorbia antiquorum* L. and *Euphorbia caducifolia* Haines. Int. J. Recent Scient. Res., 4: 1-4.
21. Ashok, P.K. and K. Upadhyaya, 2012. Tannins are astringent. J. Pharmacogn. Phytochem., 1: 45-50.
22. Kolodziej, H. and A.F. Kiderlen, 2005. Antileishmanial activity and immune modulatory effects of tannins and related compounds on *Leishmania* parasitised raw 264.7 cells. Phytochemistry, 66: 2056-2071.
23. Lu, L., S.W. Liu, S.B. Jiang and S.G. Wu, 2004. Tannin inhibits HIV-1 entry by targeting gp41. Acta Pharmacol. Sin., 25: 213-218.
24. Funatogawa, K., S. Hayashi, H. Shimomura, T. Yoshida, T. Hatano, H. Ito and Y. Hirai, 2004. Antibacterial activity of hydrolyzable tannins derived from medicinal plants against *Helicobacter pylori*. Microbiol. Immunol., 48: 251-261.
25. Francisco, I.A. and M.H.P. Pinotti, 2000. Cyanogenic glycosides in plants. Braz. Arch. Biol. Technol., 43: 487-492.
26. Simin, N., D. Orcic, D. Cetojevic-Simin, N. Mimica-Dukic and G. Anackov *et al*., 2013. Phenolic profile, antioxidant, anti-inflammatory and cytotoxic activities of small yellow onion (*Allium flavum* L. subsp. *flavum*, Alliaceae). LWT-Food Sci. Technol., 54: 139-146.
27. Chudzik, M., I. Korzonek-Szlacheta and W. Król, 2015. Triterpenes as potentially cytotoxic compounds. Molecules, 20: 1610-1625.
28. Weston, L.A. and U. Mathesius, 2013. Flavonoids: Their structure, biosynthesis and role in the rhizosphere, including allelopathy. J. Chem. Ecol., 39: 283-297.
29. Dave, H. and L. Ledwani, 2012. A review on anthraquinones isolated from *Cassia* species and their applications. Indian J. Nat. Prod. Resourc., 3: 291-319.
30. Aniszewski, T., 2007. Alkaloids-Secrets of Life: Aklaloid Chemistry, Biological Significance, Applications and Ecological Role. Elsevier Science, USA., ISBN-13: 9780444527363, Pages: 334.
31. Li, S., Q. Han, C. Qiao, J. Song, C.L. Cheng and H. Xu, 2008. Chemical markers for the quality control of herbal medicines: An overview. Chin. Med., Vol. 3. 10.1186/1749-8546-3-7.