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Many anticancer natural lead compounds fail to participate in the drug development process due to their side effects, which is more than their beneficial effect.<sup>[39]</sup> As such, any compound which has selective behavior in cancer cells, without harming normal cells will be of high significance. Triterpenoids exhibit remarkable and diverse physiological and therapeutic actions such as antiangiogenic and dedifferentiation effects that are related with anticancer actions.<sup>[40]</sup> They are even found to be a cancer reversal drug from multidrug resistant cancers.<sup>[41-43]</sup> It is interesting to note that the anticancer effects of triterpenoids have emerged as a selective apoptosis inducer in breast cancer cells while sparing normal cells.<sup>[40]</sup> In the present study, betulin followed by the new compound (29 [30]-lupene-3, 20-diol; 3  $\beta$ -form, 29-Aldehyde) exhibited a significant anticancer effect against the Hela cell line and were proven to be not toxic to normal cell. A previous study had shown that betulin was capable of selective apoptosis induction on invasive breast cancer.<sup>[44]</sup> In addition, it was found to exhibit potent antitumor potential<sup>[45]</sup> and apoptosis induction in several other cancer cells, including glioblastoma, leukemia, and lung carcinoma.<sup>[46,47]</sup> Our results in the current research are in well agreement with the literature indicating that the terpenoids as a phytochemical class with individual phytochemicals having significant anticancer potential.

## CONCLUSION

We have isolated a new and four known triterpenoids from the stem bark of *G. flavescens*, which were evaluated for immunomodulatory and cytotoxicity properties. Among all isolated and tested compounds, the new compound; 29 (30)-lupene-3, 20-diol; 3  $\beta$ -form, 29-Aldehyde (4) significantly interferes with the adaptive immune response (T-cell proliferation). It inhibited the PHA-activated T-cell with an  $IC_{50}$  of 8.7  $\mu$ g/mL.  $\beta$ -sitosterol and its glycoside form exerted moderate activity. Moreover, betulin and the new compound, respectively, exerted significant anticancer activity against the Hela cell line while  $\beta$ -sitosterol and lupeol also exhibited considerable anticancer property but with lower effect than the former two compounds.

Taking together all these results, we would like to conclude that *G. flavescens* has a good potential for future studies which might focus in other phytochemicals from the plant and animal model-based studies, which could bring more mechanistic facts about this plant activity.

## Acknowledgements

The authors would like to acknowledge the assistance of Dr. Achyut Adhikari of Central Department of Chemistry, Tribhuvan, University, Kirtipur, Kathmandu, Nepal, in the elucidation of compound structures.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

## REFERENCES

- Smith GP, Chan ES. Immunomodulating pharmaceuticals. In: Rich RR, Fleisher TA, Weyand CM, editors. Clinical Immunology Principles and Practice. Elsevier, Singapore; 2019. p. 1177-84.e1.
- Biella Cde A, Salvador MJ, Dias DA, Dias-Baruffi M, Pereira-Crott LS. Evaluation of immunomodulatory and anti-inflammatory effects and phytochemical screening of *Alternanthera tenella* Colla (*Amaranthaceae*) aqueous extracts. Mem Inst Oswaldo Cruz 2008;103:569-77.
- Ciz M, Denev P, Kratchanova M, Vasicek O, Ambrozova G, Lojek A. Flavonoids inhibit the respiratory burst of neutrophils in mammals. Oxid Med Cell Longev 2012;2012:181295.
- Adrian A, Schoppmann K, Sromicki J, Brungs S, von der Wiesche M, Hock B, *et al.* The oxidative burst reaction in mammalian cells depends on gravity. Cell Commun Signal 2013;11:98.
- Bhattacharjee S. Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transduction in plants. Curr Sci 2005 ;89:1113-21.
- Hedberg I. Flora of Ethiopia and Eritrea, in the Biodiversity of African Plants. Springer, Dordrecht; 1996. p. 802-4.
- Mohamed IET and Abdelrahman MEN, The antibacterial, antiviral activities and phytochemical screening of some Sudanese medicinal plants. EurAsian Journal of BioSciences, 2010. 4: p8-16.
- Prakash L, Singh R. Chemical examination of *Grewia flavescens*. Pharmazie 1981;36:576.
- Yanadaiah J. Assessment of antidiabetic activity of ethanol extract of *Grewia flavescens* Juss leaves against alloxan induced diabetes in rats. J Glob Trends Pharm Sci 2013;4:1086-90.
- Chauke MA, Shai LJ, Mogale MA, Tshisikhawe MP, Mokgotho MP. Medicinal plant use of villagers in the Mopani district, Limpopo province, South Africa. Afr J Tradit Complement Altern Med 2015;12:9-26.
- Elhassan G, Yagi S. Nutritional composition of *Grewia* species (*Grewia tenax* (Forsk.) Fiori, *G. flavescens* Juss and *G. villosa* Willd) fruits. Adv J Food Sci Technol 2010;2:159-62.
- Ovesná Z, Vachálková A, Horváthová K, Tóthová D. Pentacyclic triterpenoic acids: New chemoprotective compounds. Minireview. Neoplasma 2004;51:327-33.
- Liby KT, Yore MM, Sporn MB. Triterpenoids and rexinoids as multifunctional agents for the prevention and treatment of cancer. Nat Rev Cancer 2007;7:357-69.
- Ríos JL. Effects of triterpenes on the immune system. J Ethnopharmacol 2010;128:1-4.
- Singh S, Yadav AK. Evaluation of immunomodulatory activity of *Grewia asiatica* in laboratory animals. J Chem Pharm Res 2014;6:2820-6.
- Marya B, Dattani KH, Patel DD, Patel PD, Patel D, Suthar MP, *et al.* In vitro cytotoxicity evaluation of aqueous fruit and leaf extracts of *Grewia asiatica* using MTT assay. Pharm Chem 2011;3:282-7.
- Wiik P, Opstad PK, Bøyum A. Granulocyte chemiluminescence response to serum opsonized zymosan particles *ex vivo* during long-term strenuous exercise, energy and sleep deprivation in humans. Eur J Appl Physiol Occup Physiol 1996;73:251-8.
- Demirkiran O, Ahmed Mesaik M, Beynek H, Abbaskhan A, Iqbal Choudhary M. Cellular reactive oxygen species inhibitory constituents of *Hypericum thasium* Griseb. Phytochemistry 2009;70:244-9.
- Yeskaliyeva B, Mesaik MA, Abbaskhan A, Kulsoom A, Burasheva GSh, Abilov ZH, *et al.* Bioactive flavonoids and saponins from *Climacoptera obtusifolia*. Phytochemistry 2006;67:2392-7.
- Helfand SL, Werkmeister J, Roder JC. Chemiluminescence response of human natural killer cells. I. The relationship between target cell binding, chemiluminescence, and cytolysis. J Exp Med 1982;156:492-505.

21. Böyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand J Clin Lab Invest Suppl* 1968;97:77-89.
22. Nielsen M, Gerwien J, Geisler C, Röpke C, Svejgaard A, Odum N. MHC class II ligation induces CD58 (LFA-3)-mediated adhesion in human T cells. *Exp Clin Immunogenet* 1998;15:61-8.
23. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;65:55-63.
24. Dimas K, Demetzos C, Marsellos M, Sotiriadou R, Malamas M, Kokkinopoulos D. Cytotoxic activity of labdane type diterpenes against human leukemic cell lines *in vitro*. *Planta Med* 1998;64:208-11.
25. Wenkert E, Baddeley GV, Burfitt IR, Moreno LN. Carbon-13 nuclear magnetic resonance spectroscopy of naturally-occurring substances-LVII Triterpenes related to lupane and hopane. *Organ Mag Reson* 1978;11:337-43.
26. Gupta MM, Verma RK. Lipid constituents of *Cissus quadrangularis*. *Phytochemistry* 1991;30:875-8.
27. Akihisa T, Takamine Y, Yoshizumi K, Tokuda H, Kimura Y, Ukiya M, *et al.* Microbial transformations of two lupane-type triterpenes and anti-tumor-promoting effects of the transformation products. *J Nat Prod* 2002;65:278-82.
28. Saleem M. Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. *Cancer Lett* 2009;285:109-15.
29. Babu S, Jayaraman S. An update on  $\beta$ -sitosterol: A potential herbal nutraceutical for diabetic management. *Biomed Pharmacother* 2020;131:110702.
30. Laavola M, Haavikko R, Hämäläinen M, Leppänen T, Nieminen R, Alakurtti S, *et al.* Betulin derivatives effectively suppress inflammation *in vitro* and *in vivo*. *J Nat Prod* 2016;79:274-80.
31. Dodeigne C, Thunus L. Chemiluminescence as diagnostic tool. A review. *Talanta* 2000;51:415-39. Available from: <https://pubmed.ncbi.nlm.nih.gov/18967873/>. [Last accessed on 2021 May 08]
32. Moon JJ, Suh H, Bershteyn A, Stephan MT, Liu H, Huang B, *et al.* Interbilayer-crosslinked multilamellar vesicles as synthetic vaccines for potent humoral and cellular immune responses. *Nat Mater* 2011;10:243-51.
33. Khan U, Ghazanfar H. T lymphocytes and autoimmunity. *Int Rev Cell Mol Biol* 2018;341:125-68.
34. Desai F, Ramanathan M, Fink CS, Wilding GE, Weinstock-Guttman B, Awad AB. Comparison of the immunomodulatory effects of the plant sterol beta-sitosterol to simvastatin in peripheral blood cells from multiple sclerosis patients. *Int Immunopharmacol* 2009;9:153-7.
35. Bouic PJ, Etsebeth S, Liebenberg RW, Albrecht CF, Pegel K, Van Jaarsveld PP. Beta-sitosterol and beta-sitosterol glucoside stimulate human peripheral blood lymphocyte proliferation: implications for their use as an immunomodulatory vitamin combination. *Int J Immunopharmacol* 1996;18:693-700.
36. Bin Sayeed MS, Karim SM, Sharmin T, Morshed MM. Critical analysis on characterization, systemic effect, and therapeutic potential of beta-sitosterol: A plant-derived orphan phytosterol. *Medicines (Basel)* 2016;3:29.
37. Assmann G, Cullen P, Erbey J, Ramey DR, Kannenberg F, Schulte H. Plasma sitosterol elevations are associated with an increased incidence of coronary events in men: Results of a nested case-control analysis of the Prospective Cardiovascular Münster (PROCAM) study. *Nutr Metab Cardiovasc Dis* 2006;16:13-21.
38. Bin Sayeed MS, Karim SM, Sharmin T, Morshed MM. Critical analysis on characterization, systemic effect, and therapeutic potential of beta-sitosterol: A plant-derived orphan phytosterol. *Medicines (Basel)* 2016;3:29.
39. Wang J, Jiang YF. Natural compounds as anticancer agents: Experimental evidence. *World J Exp Med* 2012;2:45.
40. Ghante MH, Jamkhande PG. Role of pentacyclic triterpenoids in chemoprevention and anticancer treatment: An overview on targets and underlying mechanisms. *J Pharmacopuncture* 2019;22:55-67.
41. Yan XJ, Gong LH, Zheng FY, Cheng KJ, Chen ZH, Shi Z. Triterpenoids as reversal agents for anticancer drug resistance treatment. *Drug Discov Today* 2014;19:482-8. Available from: <https://pubmed.ncbi.nlm.nih.gov/23954181/>. [Last accessed on 2021 May 08].
42. Bishayee A, Ahmed S, Brankov N, Perloff M. Triterpenoids as potential agents for the chemoprevention and therapy of breast cancer. *Front Biosci (Landmark Ed)* 2011;16:980-96.
43. Lima LM, Perazzo FF, Carvalho JCT, Bastos JK. Anti-inflammatory and analgesic activities of the ethanolic extracts from *Zanthoxylum riedelianum* (Rutaceae) leaves and stem bark. *J Pharm Pharmacol* 2007;59:1151-8. Available from: <https://pubmed.ncbi.nlm.nih.gov/17725859/>. [Last accessed on 2021 May 08].
44. Sudhakar V, Ashok Kumar S, Varalakshmi P, Sujatha V. Protective effect of lupeol and lupeol linoleate in hypercholesterolemia associated renal damage. *Mol Cell Biochem* 2008;317:11-20.
45. Apan AAMTR, Castorena ALP, Romo de Vivar A. Anti-inflammatory constituents of *Mortonia greggii* Gray. *Z für Naturforsch C* 2004;59:237-43. Available from: <https://pubmed.ncbi.nlm.nih.gov/15241934/>
46. Cháirez-Ramírez MH, Moreno-Jiménez MR, González-Laredo RF, Gallegos-Infante JA, Rocha-Guzmán NE. Lupane-type triterpenes and their anti-cancer activities against most common malignant tumors: A review. *EXCLI J* 2016;15:758-71.
47. Mullauer FB, Kessler JH, Medema JP. Betulin is a potent anti-tumor agent that is enhanced by cholesterol. *PLoS One* 2009;28;4:e1.