IN VITRO CYTOTOXIC EVALUATION OF *Sorbaria tomentosa*

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ABSTRACT

Locally famous Karhee or Berre [*Sorbaria tomentosa* (Lindl.) Rehder] exhibits medicinal value as a large woody shrub. The present study examined the cytotoxic activities of *S. tomentosa* using methanolic extracts and fractions (*n*-hexane, dichloromethane, ethyl acetate and water) against three cancer cell lines (lung A-549, hepatocellular HepG2and urinary bladder EI-138). Cytotoxic assays were carried out with five concentrations (0.05, 0.01, 0.05, 0.1 and 0.5 mg mL^{-1}) of methanolic extract and its subfractions through MTT assay [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide]. Results revealed *n*-hexane and ethyl acetate fraction being the most potent against all test cancer cell lines with higher IC50 values. Both fractions also exhibited the maximum reduction in the cell viability in dose dependent manner. Preliminary results suggest the promising anticancer potential of n-hexane and ethyl acetate *S. tomentosa* against lung A-549, hepatocellular HepG2and urinary bladder EI-138 cell lines. Further studies are required to know the mechanism(s) involved in the cell death.

Key words: Anticancer activity, Cytotoxicity, Karhee, *Sorbaria tomentosa*

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INTRODUCTION

For millennia, natural products have been used in treating different diseases (Ogbole *et al*., 2017), and in recent time, 70% of the drugs are models of natural products (Newman and Cragg, 2016), while 80% of people in developing nations still rely on herbal medicines as main source of health care (Ekor, 2014). During 1981 to 2010, around 700 natural products or natural product derived from New Chemical Entities were approved (Ogbourne and Parsons, 2016). In spite of improved treatment options, cancer is still the second reason for death globally (Fitzmaurice *et al*., 2017). Natural products are known to contain compounds for cancer chemo-preventive agents, as illustrated in the discovery of the vinca alkaloids (vincristine and vinblastine), taxols (paclitaxel and docetaxel), camptothecin and etoposide (Wall and Wani, 1996). In the quest for new therapeutic or preventative modalities, anticancer properties of the indigenous medicinal plants still need to be explored.

Rosaceae is a medium-sized family of flowering plants, having 4828 species and 91 genera. It is also called the rose family, and it contains many natural products which are used against many human and animal ailments (Christenhusz *et al*., 2016). It also contains various edible fruits which are economically very significant (Watson *et al*., 1992). Several reports have claimed the genus *Sorbaria* of this family holds antioxidant activity, thus can be used for the treatment of cancer and chronic liver damage (Zhang *et al*., 2007; Jiwon Jang *et al*., 2020). It was also revealed that members of the genus *Sorbaria* exhibit anti-proliferative, anti-inflammatory, hepatoprotective, anti-photoaging and antimelanogenic activities (Jang *et al*., 2020; Nishi *et al*., 2020; Hongxi *et al*., 2021). The genus contains four species, all of which are wild having medicinal as well as ornamental values. *Sorbaria tomentosa* (Lindl.) Rehder commonly known as Hamalayan Sorbaria is a wild spreading, deciduous shrub, native to Pakistan, and is also present in Afghanistan, Tajikistan, Korea, China, northern India and Nepal. Its habitat is usually cooler, often grows in water channels and at top positions from 2,100-2,700 meters (Gamble, 1972). Its flowering period spreads from March to May and fruiting starts from June to August (Bibi *et al*., 2021). It is grown as an ornamental plant as well as barrier plantings in the Himalayas to keep animals out of fields and gardens. This weed tolerates atmospheric pollution, the flowers and leaves are used in both conventional and traditional medicines, stem and fruit are used in asthma treatment and in a variety of ailments (Pankaj *et al*., 2013). Its inflorescence is mixed with mustard oil and used as an antiseptic agent to cure skin rashes of newly born babies due to the occurrence of gallic acid and tannins (Hamayn *et al*., 2006). The paste of the flower is mixed with milk to treat wounds and burns (Mahesh Kumar *et al*., 2009). The whole plant has been recommended for the treatments of different diseases (Rahman *et al*., 2016).

There is less work done on the cytotoxic activity of *S. tomentosa* with respect to other species of *Sorbaria*, so there is a need to explore the cytotoxic potential of *S. tomentosa*. In the present investigation, methanolic extract and fractions (*n* hexane, dichloromethane, ethyl acetate and water) of the whole plant of *S. tomentosa* were evaluated for their cytotoxic activity against three human cancer cell lines A-549 (lung adenocarcinoma), HepG2 (hepatocellular carcinoma) and EI-138 (urinary bladder cancer cell) by MTT assay.

MATERIAL AND METHODS

Plant material and fractionation

Whole plant of *S. tomentosa* (No. GC. Bot. Herb. 816) was collected from the Northern area, Kalam, Pakistan, dried in shade and crushed into fine powder. The ground material (2 kg) was macerated in 95% methanol, filtered paper (Whatman 42), and the filtrate was concentrated to dryness at 50 $^{\circ}$C using a

rotary evaporator. The crude extract (180 g) was suspended in distilled water (200 mL) in a separating funnel and partitioned with *n*-hexane, dichloromethane, and ethyl acetate. About 21, 28, 35 and 26 g of the solid extract were weighted from *n*-hexane, dichloromethane, ethyl acetate, and water, respectively.

Cytotoxic activity by MTT assay

Methanolic extract and different subfractions (hexane, dichloromethane, ethyl acetate and water) of methanolic extract of *S. tomentosa* were evaluated for their cytotoxicity activity through MTT assay [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide] against three tumor cell lines viz., A-549, HepG2 and EI-138 (acquired from European Collection of Authenticated Cell Cultures, Salisbury, UK) following protocols of Mosmann *et al*. (1983). RPMI-1640 medium amended with fetal bovine serum (10%) was used to cultivate these cell lines.

For cytotoxicity experiment, five different concentrations (0.05, 0.01, 0.05, 0.1 and 0.5 mg mL^{-1}) of the methanolic extract as well as for each fraction were prepared. The experiment was comprised of 90 treatments, each extract/fraction consisted of 30 treatments with each cell line.

For the experiment, single cell suspension of a tumor cell in logarithmic growth phase was seeded in 96-well plates at 1 mL $well^{-1}$ (1×10^3 cells mL^{-1}) in quadruplet. The plates were incubated overnight at 37 °C for adherence of monolayer to the wells. After incubation, media was replaced with 1 mL of each concentration (0.05, 0.01, 0.05, 0.1 and 0.5 mg mL^{-1}) of the methanolic extract/fraction. After another 24 hours, the methanolic extract/fraction was replaced with 1 mL of MTT dye reagent and kept for incubation for 48 hours at 37 °C. After removing MTT, isopropanol was added to each well. Control, received only growth media. Using a microplate reader absorbance of the samples was recorded at 570 nm.

Data analysis

The experiment was conducted in a completely randomized design. Means, standard deviations (SD) and standard error (SE) were calculated on excel. ANOVA followed by Fisher's protected least significant difference test ($P \leq 0.05$) was used to determine the significant effects ($P < 0.05$) among the treatments using the SATISTIX 8.1.

RESULTS AND DISCUSSION

The cytotoxic potential of different concentrations of methanolic extract and sub fractions (*n*-hexane, dichloromethane, ethyl acetate and water) of *S. tomentosa* has been assessed using MTT assay in three cancer cell lines (A-549, HepG2 and EI-138). Ethyl acetate sub fraction found to be more cytotoxic (IC_{50}: 0.08, 0.10 and 0.06 mg mL^{-1}) followed by *n*-hexane fraction (IC_{50}: 0.09, 0.01 and 0.1 mg mL^{-1}) towards three cancer cell line (A-549, HepG2 and EI-138, respectively) as compared to the control (Table 1). The IC_{50} of the methanolic, dichloromethane, and water fractions were significantly low (Table 1). Generally, A549 and EI-138 were more sensitive to higher concentrations, i.e. 0.1 and 0.5 mg L^{-1} of the methanolic extract and its sub fractions, while HepG2, exhibited the greater sensitivity to the highest used concentration of 0.5 mg L^{-1} of *n*-hexane and ethyl acetate fraction only. However, percentage viability of all three cancer cell lines was minimum in *n*-hexane followed by ethyl acetate sub fraction (Fig. 1 A-C). In this regard, the cells viability in A549, HepG2 and EI-138 were significantly declined to 25-32%, 43-55 and 24-41%, respectively at 0.1 mg L^{-1} with *n*-hexane or ethyl acetate as compared to the control. However, at 0.5 mg L^{-1}, the cells viability showed the maximum reduction in all three cancer cell lines, where cell viability was reached to 4.0-6.0% and 19-24% with *n*-hexane and ethyl acetate, respectively (Fig. 1 A-C).

Difference in cytotoxic response of cancer cell towards different sub fractions might be due to the difference in the presence of the bioactive compounds as reported previously (Al-Sheddi, 2019). Khasawneh *et al.* (2015) also documented hexane fraction of *Leptadenia pyrotechnica* as the most potential in decreasing cell viability in a dose and time-dependent manner, and they correlated the anticancer activity with the presence of active compounds in the extract. Chloroform and ethyl acetate extracts obtained from aerial parts of *Rhazya stricta* displayed considerable cytotoxic activity (LC_{50}: 18.1 and 13.9 µg mL^{-1}, respectively) against HepG2 and colon cancer cells (CaCo) (Phondani *et al*., 2016). Bin Rohin *et al*. (2017) found significant cytotoxic effects and morphological alterations in human glioblastoma cell line (U-87) due to the effect of phenolic contents in ethyl acetate extract of *Vernonia amygdalina*. Usmani *et al*. (2018) investigation showed bioactive compounds in the extract of *Cordia dichotoma* had anti-proliferative potential on human cervix epitheloid (HeLa) and human lung (A549) carcinoma cells by employing ROS generation, and by causing detachment, aggregation and death of the cell. Therefore, anticancer potential of *n*-hexane and ethyl acetate fractions reported in the present study may be ascribed to the occurrence of a large number of diverse bioactive compounds, e.g. polyphenols, flavonoids and brassinosteroids. These compounds may induce apoptosis; antioxidant activity, target specificity and cancer cell cytotoxicity (Farshori *et al*., 2014; Greenwell and Rahman, 2015). Moreover, in the present study, the solvent utilized may also be suitable to extract bioactive compounds of anticancer activity in *S. tomentosa* (Ali *et al*., 2018).

CONCLUSIONS

The results showed that that *n*-hexane and ethyl acetate fractions of *S. tomentosa* were more cytotoxic as compared to methanolic extract, dichloromethane fraction and water fraction. The cell viability decreased significantly at higher concentrations of the extract fraction. Further, biochemical studies are needed to validate the apoptotic efficacy and to explore the mechanism(s) of cell death associated with this process.

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Table 1: Cytotoxic activity (IC_{50} values) of *Sorbaria tomentosa* extract/fractions against different human cancer cell lines lung A-549, hepatocellular HepG2 and urinary bladder EI-138.

***S. tomentosa* Fractions**	**IC_{50} values (mg/ mL)**		
	A-549	**HepG2**	**EI-138**
Methanolic extract	0.37 **C** ± 2.0	0.25 **C** ± 0.7	0.15 **BC** ± 1.8
n-hexane fraction	0.09 **A** ± 1.4	0.01 **A** ± 1.0	0.11 **B** ± 1.4
Dichloromethane fraction	0.60 **D** ± 1.7	0.35 **D** ± 2.0	0.37 **E** ± 2.0
Ethyl acetate fraction	0.08 **A** ± 2.3	0.10 **B** ± 1.4	0.06 **A** ± 3.5
Water Fraction	0.15 **B**± 1.8	0.31 **D** ± 1.4	0.25 **D** ± 1.1

± indicate standard errors of mean of three replicates. Values with different letters show significant difference ($P \leq 0.05$) as determined by LSD-test.

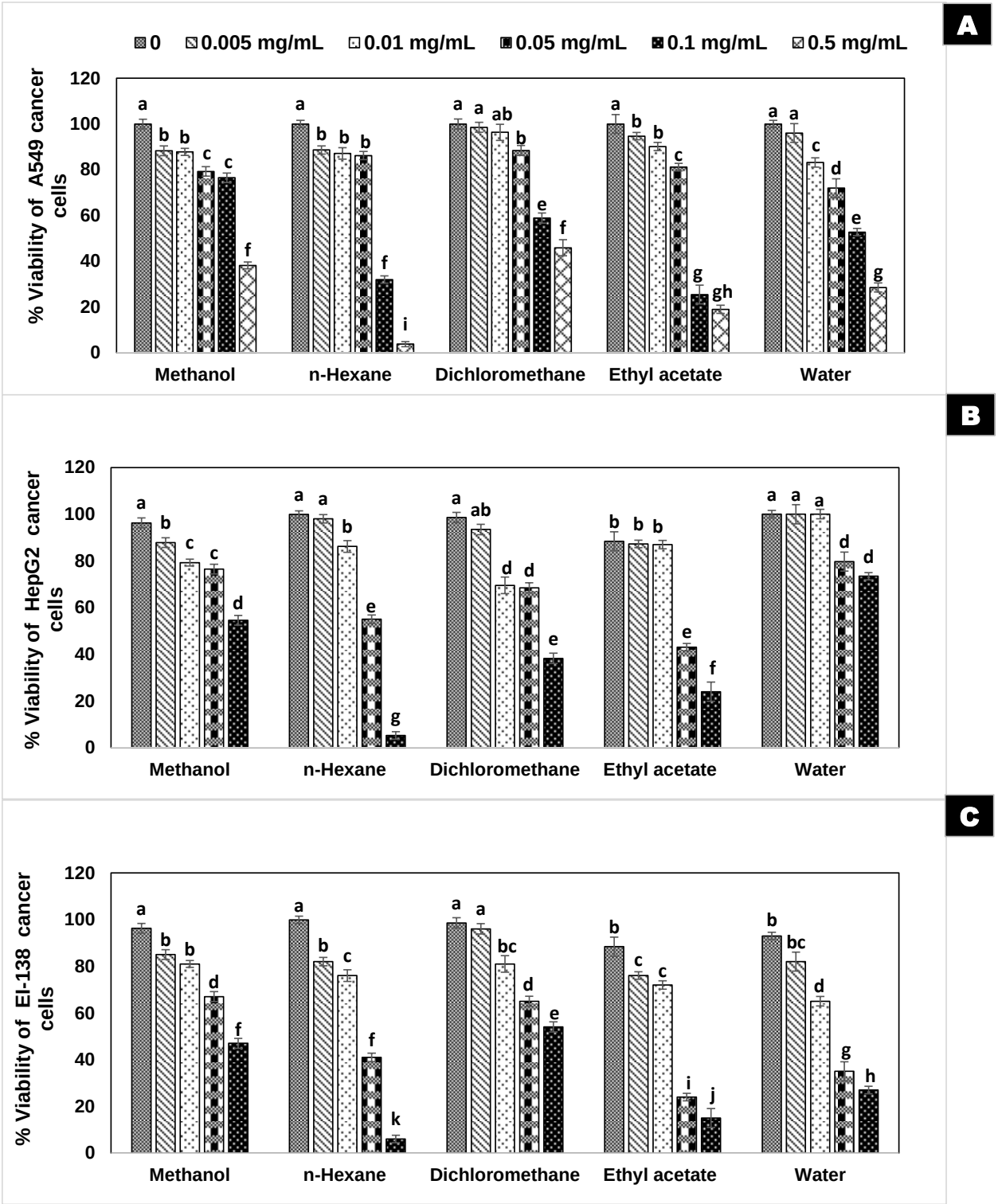

Fig. 1 (A-C): Cytotoxic activity of *Sorbaria tomentosa* against human cancer cell lines lung A-549 (A), hepatocellular HepG2 (B) and urinary bladder EI-138 (C). Error bars indicate standard errors of mean of three replicates. Values with different letters show significant difference ($P \leq 0.05$) as determined by LSD-test.