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In-vitro cytotoxic effects of *Abutilon theophrasti* from north western Himalayas against the cell lines of liver cancer (HepG2)

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SUMMARY

Liver cancer i.e. hepatocellular carcinoma is the most frequent type of primary liver cancer in adults and the most common cause of death in people with cirrhosis. Epidemiological studies indicate that liver cancer is associated with hypoglycemia, erythrocytosis, hypercalcemia, hypercholesterolemia, and diarrhea. Natural means such as herbs, spices, and alternative agents control the development of cancer through their antioxidant activity. In the present study, *Abutilon theophrasti* (crude extracts) was evaluated against the cell lines of liver cancer (HepG2). MTT assay was used to evaluate anticancer activities from extracts. Among all selected parts (root, leaf, stem, and fruit body) only root showed potential anticancer activity. The IC_{50} value of methanolic root extract (R-Mα) was found to be 402.25µg/ml and for hexane root extract (R-Hα) it was 473.96 µg/ml. The activity can be related to phytoconstituents like flavonoids, catechins, and phenolics.

Keywords: *Abutilon theophrasti*, Anti-cancerous, Hepatocellular, MTT Assay

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INTRODUCTION

Hepatocellular carcinoma is the most prime hepatic malignancy with survival rates ranging from 6 to 20 months (Waller *et al.*, 2015). It is now the world's sixth most common cancer, contributing to the third leading cause of cancer related fatality. The interaction of environmental and genetic factors leads to the development of Hepatocellular carcinoma (Waller *et al.*, 2015). Non-alcoholic steatohepatitis, along with hepatitis B virus, hepatitis C virus infection, and excessive alcohol consumption is thought to be an important risk factor for HCC development (Gomes *et al.*, 2013). It was thought that after Beasley linked hepatitis B infection to the development of HCC, the root cause had been discovered (Beasley *et al.*, 1981). Following that, more research linked “Hepatocellular carcinoma” to other underlying cirrhosis etiologies (Cucchetti *et al.*, 2009). Ongoing research has linked metabolic syndrome to cancer risk. Fibrogenesis and inflammation predispose the liver to dysplasia and, as a result, cancer (Block *et al.*, 2003).

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According to Cancer Research UK, approximately 17 million new cases were reported in 2018, with 9.6 million fatalities. If current trends continue, there will be more than 27 million patients of cancer by 2040 (Ashraf, 2020). Anticancer drugs can now be targeted at controlling the fundamental mechanisms within cells (Bai *et al.*, 2017). In recent years, there has been a search for an anticancer drug from plant bioactive compounds, in addition to customary anticancer medicine, which are time-consuming and expensive. Furthermore, when compared to synthetic compounds, drug discovery and development using plant-based compounds is less costly (Rayan *et al.*, 2017). Plants produce a variety of secondary metabolites, which are primarily used for defence and reaction to ecology cues like abiotic and biotic stress (Ashraf, 2020). Many plant-derived substances (taxol, vinblastine, topotecan, and others) have been shown to be effective against cancer in clinical trials (Slichenmyer and Von Hoff, 1991; Brahmer and Ettinger, 1998; Umair *et al.*, 2017; Umair *et al.*, 2019; Hassan *et al.*, 2021b).

Abutilon theophrasti, a Malvaceae member native to Asian tropical countries, has been used for centuries for its wind-repelling, detoxicating, and dieresis properties (Tian *et al.*, 2017). The leaves of *Abutilon theophrasti* are commonly used to treat ulcers, swelling, and venom (Hassan *et al.*, 2016; Hassan *et al.*, 2021a). *Abutilon theophrasti* leaves have anti-inflammatory and analgesic attributions (Su *et al.*, 2010), also do possess potential to get against arthrosis, rheumatic pains, dysentery, bruises, sprains, tinnitus, deafness and otitis media (Dinu *et al.*, 2010; Balah and Nassar, 2011). The prime chemical compounds extracted from the plant include flavonoids and phenolic acids (Sikorska and Matlawska, 2008; Tian *et al.*, 2012). Phenolic acids and Flavonoids are thought to have a variety of pharmacological properties, including antibacterial, antioxidant, anti-inflammatory, hepatoprotective, and anticancer. The valley of Kashmir present in the bosom of Himalayas is known as the paradise on the earth. The area is inhabited with diverse flora (Sajad *et al.,* 2021) having gastronomic and medicinal attributions. The local people do possess the faith in the endemic flora for the treatment of various health disorders (Mir *et al.,* 2021; Haq *et al.,* 2022a; Haq *et al.,* 2022b), the area has an important traditional medicinal system from centuries (Mir *et al.,* 2021) not only for humans but also for their live stock (Farooq *et al.,* 2022). In the present study, we obtained the plant form the valley (Kashmir), analysed its crude extracts against hepatocellular carcinoma cell lines (HepG2).

MATERIAL AND METHODS

PLANT DISTRIBUTION AND COLLECTION

Abutilon theophrasti (Figure 1A) is a dicot that belongs to the family Malvaceae. The plant is an annual and exotic species. Native distribution ranges include Temperate Asia, Tropical Asia, Africa, and Europe. In Jammu and Kashmir we collected the said plant species from the administrative district “Baramulla” (Figure 2). The key locations were Gulmarg, Bandipora, and Hamrae. The plant (*Abutilon theophrasti*) was identified and registered “VS-No.2113-KASH” (Figure 1B) at the “Herbarium Centre for Biodiversity and Taxonomy, University of Kashmir” India.

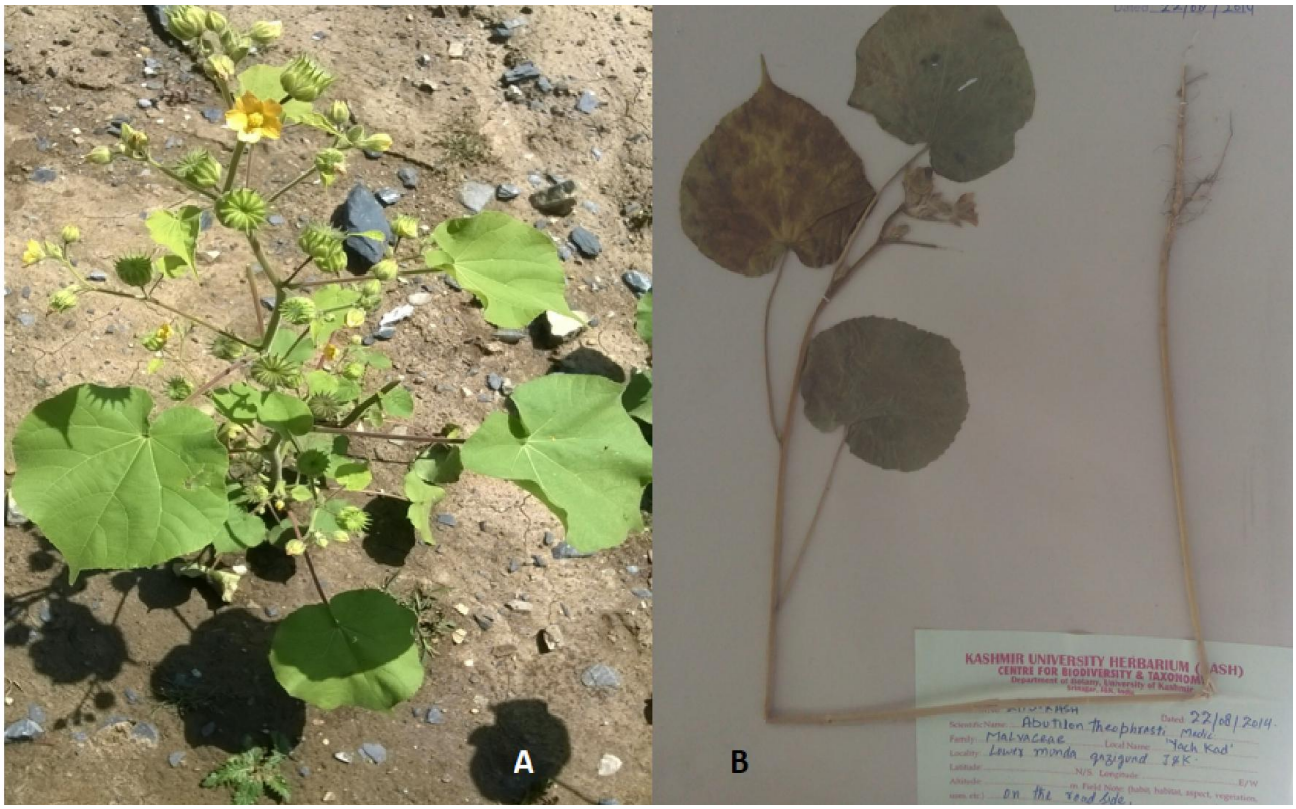

Figure 1: (A) *Abutilon theophrasti* (velvetleaf) (B) Herbarium.

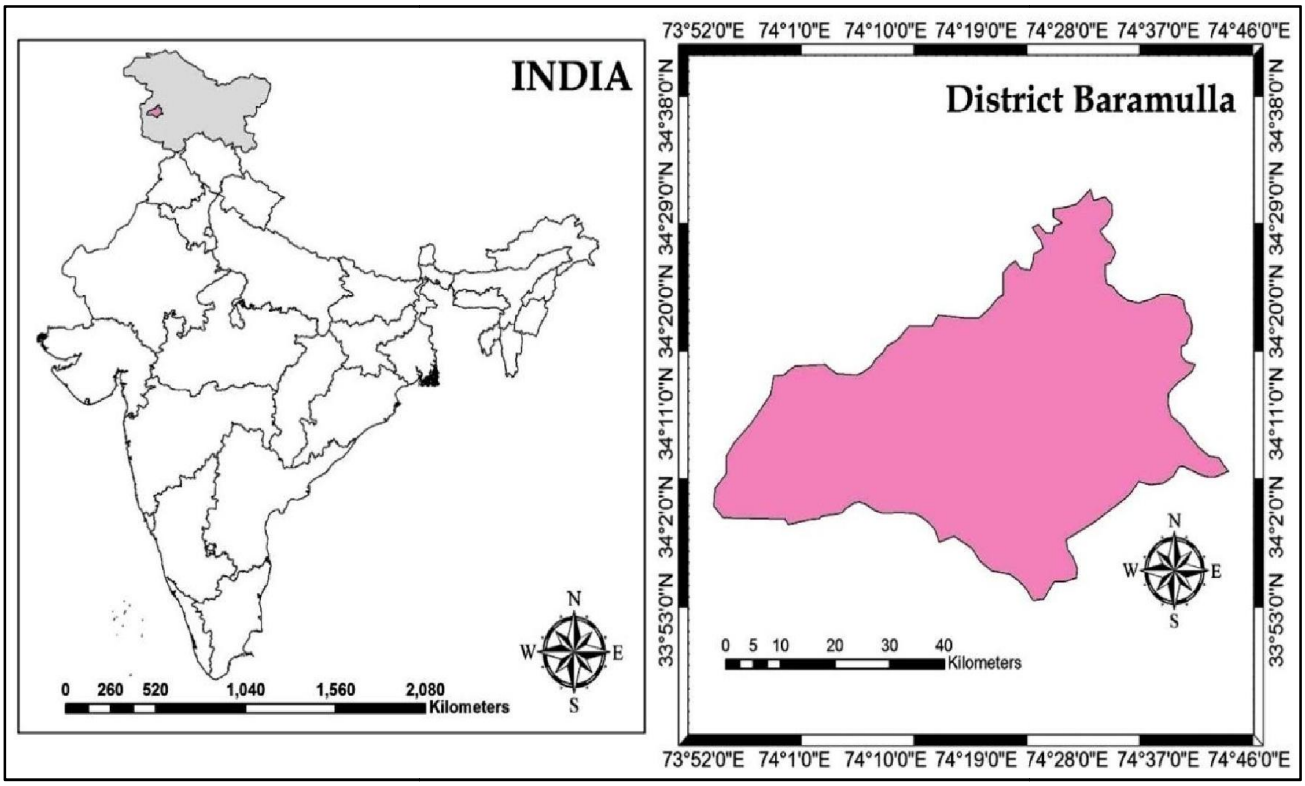

Figure 2: Map of collection sites.

PREPARATION OF EXTRACTS

For about 15 days, the selected plant material was shade dried at room temperature. Before extraction, dried plant samples were powered, sieved to fine mesh, and stored in polyethylene bags at "room temperature". The solvents utilized were "methanol" and "hexane". The extraction was accomplished through a simple maceration procedure (Hassan *et al.*, 2021b). The extracts were concentrated to dryness using a "rotary evaporator". Obtained extracts were given specific codes like (methanolic root extract as R-Mα, methanolic leaf extract as L-Mβ, methanoic stem extract as S-Mγ, methanolic fruitbody extract as F-Mδ, hexane root extract as R-Hα, hexane leaf extract as L-Hβ, hexane stem extract as S-Hγ and hexane fruitbody extract as F-Hδ.

MTT ASSAY

The MTT assay is a standard colorimetric assay that measures colour changes to determine the activity of enzymes that convert MTT to formazan, which results in a purple colour (Patel *et al.*, 2009). Reduction takes place in the mitochondria of the cell in presence of reductase enzymes, so the conversion can be used as a measure for viable (living) cells. The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) cytotoxicity assay was used on hepatocellular carcinoma cell lines (HepG2). In a 96-well plate (200L/Well), 104 cells per well were seeded. After a 24-hour incubation period, cells were treated with various concentrations (100g/ml, 200g/ml, 300g/ml, 400g/ml, 500g/ml, 600g/ml) of *Abutilon theophrasti* extracts, such as hexane leaf extracts (L-H), methanolic stem extract (S-H), hexane root extract (R-H), hexane fruit body extract (After 48 hours of incubation, the extract was removed from the old medium and replaced with 20 litres of MTT solution (5 mg/ml in PBS) in a new medium. Finally, formazan crystals were dissolved in DMSO (150L) and read at 570 nm using a plate reader from I Mark Elisa. Using the formula below, the percentage of viable cells was calculated

$$\text{Percentage of viable cells} = \frac{mean\ absorbance\ of\ the\ sample}{mean\ absorbance\ of\ the\ control} \times 100$$

STATISTICAL ANALYSIS

ANOVA, box plots and correlation were performed by using the PAST software ver.3.14 (West *et al.*, 2020) to check the significance level of hexane and methanolic extracts against HepG2 cell lines.

RESULTS AND DISCUSSION

The MTT assay revealed that only methanolic and hexane root extracts (R-Mα, R-Hα) were cytotoxic on HepG2 cells in all tested extracts. The MTT assay result for 48 hrs incubation of crude drug at the concentrations "100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, 500µg/ml, and 600µg/ml" showed decreased HepG2 cell viability as the concentration got increased (Figure 3, Figure 4). Both methanolic and hexane root extracts showed significant variation ($x^{2=}$ 157.78 df= 18.31 p = 0.005) in the cytotoxic effects on HepG2 cell lines. The effect of Hexane extract is correlated with a "p-value of 0.07", however, the methanolic extract is correlated with p =0.08.The IC_{50} value of Methanolic root extracts (R-Mα) was 402.25 along with this hexane root

extracts (R-Hα) displayed IC_{50} value of 473.96 was observed. The mean effect of both (methanolic, hexane) extracts on cell viability is shown in Figure 5.

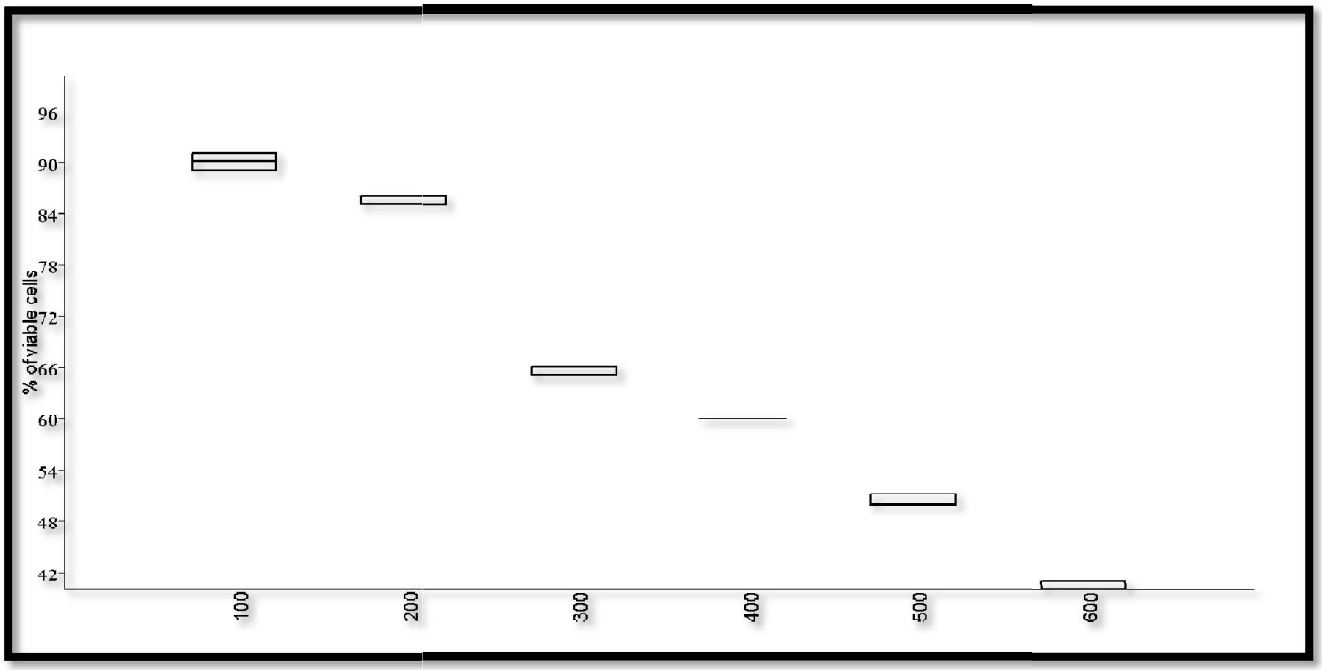

Figure 3: Cytotoxic effects of Hexane extracts of *Abutilon theophrasti* against HepG2.

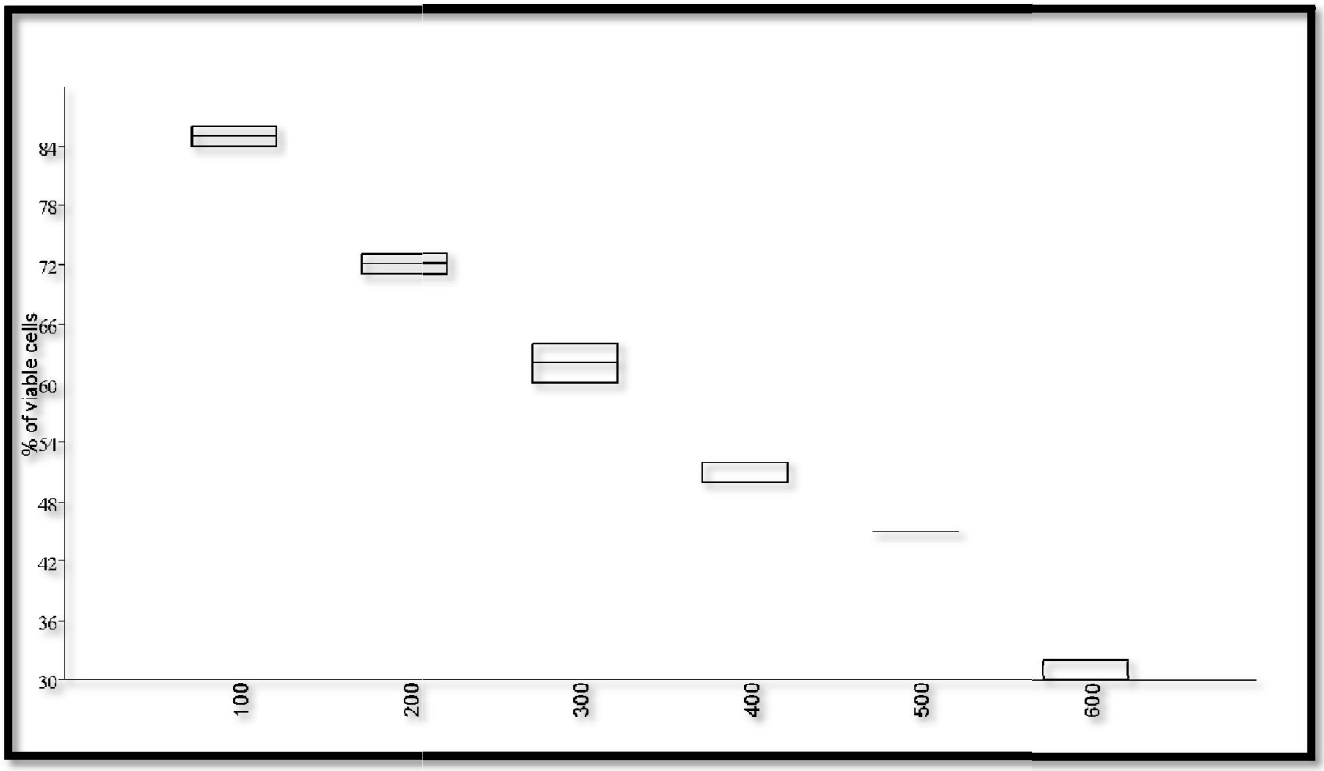

Figure 4: Cytotoxic effects of Methanolic extracts of *Abutilon theophrasti* against HepG2.

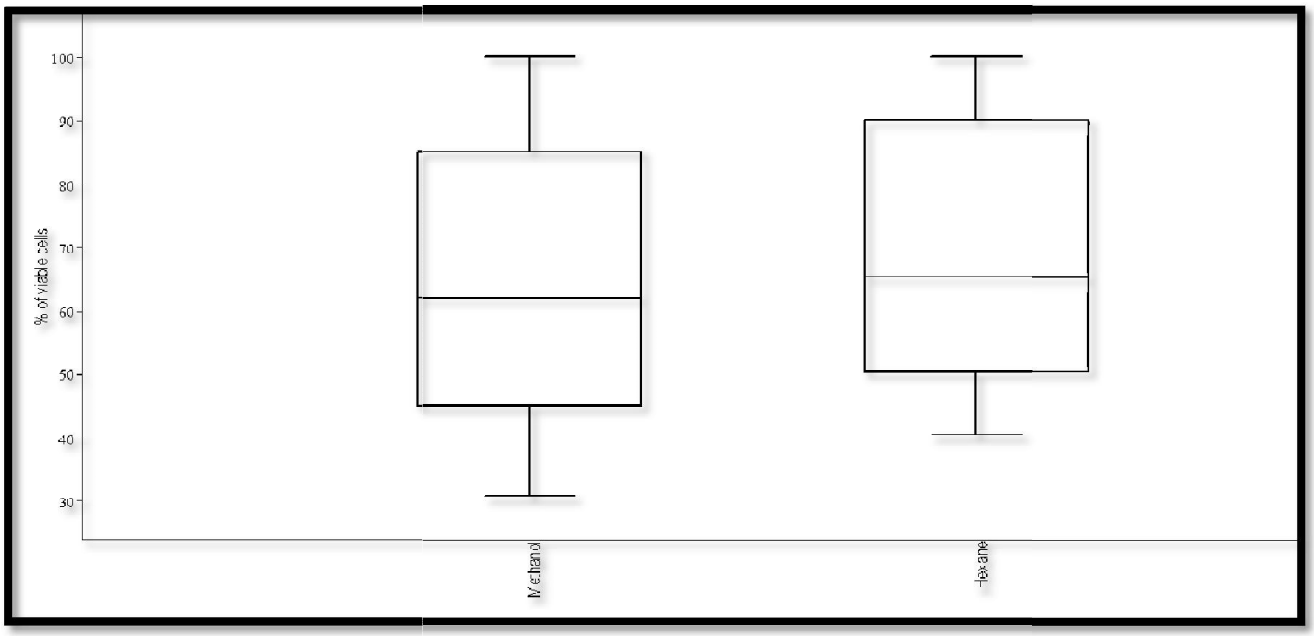

Figure 5: Comparative cytotoxic effects of Methanolic and hexane extracts of *Abutilon theophrasti* against HepG2.

Flavonoid glycosides, catechins, anthocyanidins, sterols, triterpenes, tannins, vitamins, sugars, and other phytochemicals have been discovered in *Abutilon theophrasti* (Paszkowski and Kremer, 1988; Dinu *et al.*, 2010; Balah and Nassar, 2011; Tian *et al.*, 2012). These phytoconstituents can be attributed to the cytotoxic effects of our extracts, as we know that flavonoids and phenolic have many pharmacological activities, such as antibacterial action, antioxidant, anti-inflammatory, hepatoprotective activity and anticancer (Zheng *et al.*, 2007; Cushnie and Lamb, 2011). Our study is further supported by the researchers (Mamadalieva *et al.*, 2014). Tashkent, Uzbekistan confirms that aerial parts of *Abutilon theophrasti* (leaf, flower/seed) have cytotoxic effects on MCF-7, CCRF-CEM, and CEM/ADR5000 cancer cells**,** meanwhile our studies didn't find any cytotoxic activity of aerial parts of the *Abutilon theophrasti* against HepG2**.** This could be due to the variation in phytoconstituents as our selected plant's (*Abutilon theophrasti*) collection site (Qazi Gund, Lower Munda) is different. Due to geographical and altitude variation phytochemistry is affected (Demasi *et al.*, 2018; Pandey *et al.*, 2018) and so is bioactivity.

CONCLUSION

Finally, the current findings provide preliminary evidence that *Abutilon theophrasti* extract has potent cytotoxic activity against HepG2 cells. More research is needed to properly assess the therapeutic potential of the *Abutilon theophrasti* to be produced as promising chemotherapeutic drugs.

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