The Pharma Innovation Journal 2018; 7(5): 305-311

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(5): 305-311

www.thepharmajournal.com
Received: 10-03-2018
Accepted: 12-04-2018

Khangembam Victoria Chanu
a) ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, Uttarakhand, India
b) Division of Animal Biochemistry, ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Leishangthem Geeta Devi
Department of Pathology, All India Institute of Medical Sciences, New Delhi, India

Sandeep Kumar Srivastava
Department of Pathology, All India Institute of Medical Sciences, New Delhi, India

Dimpal Thakuria
ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, Uttarakhand, India

Meena Kataria
Division of Animal Biochemistry, ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

AG Telang
Division of Veterinary Pharmacology, ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Correspondence
Khangembam Victoria Chanu
a) ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, Uttarakhand, India
b) Division of Animal Biochemistry, ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Phytochemical analysis and evaluation of anticancer activity of *Parkia javanica* seeds

Khangembam Victoria Chanu, Leishangthem Geeta Devi, Sandeep Kumar Srivastava, Dimpal Thakuria, Meena Kataria and AG Telang

Abstract
Parkia javanica is a species of the genus *Parkia* in the family *Mimosaceae*. Different parts of this plant are edible and also used in traditional practice of medicine. The objective of the study was to analyse the phytochemical constituents of *P. javanica* seed and evaluation of anticancer activity. Three different extracts, aqueous, ethanolic and methanolic extracts of *P. javanica* seeds were prepared and phytochemical screenings were carried out. In the extracts, compounds like alkaloids, flavonoids, phenolic compounds, saponins and anthraquinones could be detected by phytochemical analysis, thin layer chromatography and spectrophotometrically through absorption spectra. Based on the results of phytochemical assays, methanolic extract was selected for evaluation of its anticancer activity. The extract produced 50% cell death in HepG2, human liver cancer cells at 0.48 mg/mL with comparatively lesser cytotoxicity to normal cells. The present study showed that *P. javanica* seed contains bioactive compounds that are known to possess anticancer activity and the methanolic extract caused death in cancer cell at a non-toxic concentration to normal cells

Keywords: *Parkia javanica*, phytochemical analysis, methanolic extract, anticancer

Introduction
Plant-based foods not only provide essential nutrients but also contain significant amounts of bioactive compounds beneficial for health and disease prevention [1]. Presence of such phytochemicals or bioactive compounds also reasons the use of plants in traditional practice of medicine. Traditional or folk medicine is also governed by the type of vegetation available, socio-economic conditions and perspective of the ethnic group [2]. In terms of range in vegetation, north-east India covering the states of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura has the richest reservoir of plant diversity in India [3]. Till date, numerous plants used in traditional healing in the region have been reported and among them, *Parkia javanica* is one.
Parkia javanica (syn. *Parkia roxburghii*; *Parkia timoriana*) grows abundantly in the North Eastern region of India, especially in Manipur, Mizoram and Nagaland. Significant economic values of this tree species includes vegetable, medicinal and as fire wood in this region [4]. Besides consumption of this plant as one of the delicacies by the locals, the medicinal properties of this plant are also well known. Corresponding to the popularity of this plant both for consumption and medicinal values, many research have been conducted till date which led to identification of phytochemicals which may be responsible for its medicinal properties. Thioproline, a cyclic sulphur containing amino acid had been identified which is responsible for the pungent smell in seeds [5]. Anticarcinogenic effect of thioproline against squamous cell carcinoma of rats was already known [6, 7]. Similarly, there is documentation of antiproliferative effect of lectins isolated from *P.roxburghii* seeds against murine cancer cell lines [8]. Plant-based foods are also considered as a good source of antioxidants and are associated with reduced risk of cancer [9]. In our previous study, it was found that *P.javanica* seeds are rich in antioxidants [10]. Therefore, this study was undertaken with an attempt to analyse the phytochemicals of *P.javanica* seeds and to evaluate the effect of extract on cancer cell.

Materials and methods
Reagents and labwares
All the reagents used in the study were of analytical grade. The glasswares used in the study were procured from Borosil (India).

All the plastic wares including microfuge tubes, microtips used in this study were obtained from Tarson (India). For thin layer chromatography, the silica gel coated aluminium sheets (Aluchrosep silica gel 60/UV254) were obtained from S.D. Fine chemicals Ltd., Mumbai. Culture media (DMEM and RPMI) and Triton X were purchased from HiMedia. DMSO (Dimethyl Sulfoxide), PI (Propidium Iodide) and Ampicillin were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). DAPI (4′, 6-Diamidine-2 phenyl-indole-dihydrochloride) was obtained from Roche, USA. Ultrapure grade MTT was from Amresco, USA. Acqueous mountant was obtained from Vector Laboratories Inc. Burlingame, CA.

Preparation of plant extracts

Seeds of *P. javanica* were collected from Manipur, India and air dried in shade and powdered. The powdered seeds were then extracted in double distilled water, ethanol and methanol. The extraction process was repeated for three times and the extracts were pooled. The solvents were evaporated in a lyophilizer (Heto Power Dry LL3000 Freeze Dryer) to get the crude dry extracts which were used for the study. Seed pods of *P.javanica* were deposited at the herbarium of Botany Department, Bareilly College, Bareilly, Uttar Pradesh and obtained the identification number, 2011030657610A.

Phytochemical screening of the plant extracts

Phytochemical screening for the presence of alkaloids, anthraquinones, flavonoids, saponins, tannins, sterols, reducing sugars, glycosides, and terpenes in the extracts was carried following the protocol of Peach and Tracey and Radeleff [11, 12].

Spectrophotometric analysis of the extracts

The extracts were dissolved at a concentration of 0.2 mg/ml of their respective solvents used in extraction (water, ethanol and methanol) and the absorption spectra was determined at wavelength ranging from 190 to 750 nm (Spectroscan 2600 UV/Vis Spectrophotometer, Chemito) against respective solvent as blank. The peaks in the absorption spectrum of the extracts were recorded as the λmax of the phytochemicals present in the extract.

Thin layer chromatography (TLC)

Phytochemical screening of the extracts by thin layer chromatography was performed as per the methods described earlier by Wahab *et al* and Mungole *et al*. [13, 14]. Briefly, the extracts were drawn with capillary tubes and applied as spots on a stationary phase (silica-gel coated plate) about 1 cm from the base. The plate was then dipped into a suitable solvent system (mobile phase) and placed in a well-covered tank. Chromatographic tank was saturated with mobile phase at room temperature for 5 min prior to development. At the end of the chromatographic development, the separated spots were visualized under daylight and with UV light (254 nm) after spraying with chromogenic reagents at TLC documentation system (Reprostar 3, CAMAG, Switzerland). Distances between the spots were measured and the retention factor (Rf) values were recorded, using the following formula:

Rf value = (Distance moved by the compound)/(Distance moved by the solvent front)

Cell culture

Human hepatoma (HepG2) cells were gifted from the laboratory of Dr. A.K. Dinda, Department of Pathology, AIIMS, New Delhi. The cell line was maintained in monolayer culture in Dulbecco Minimum Essential Medium (DMEM) supplemented with 10% heat inactivated foetal bovine serum and 1% penicillin (10,000 IU) and streptomycin (100 µg/mL) in a humidified atmosphere of 5% CO_2 at 37 ºC in a CO_2 incubator (Binder, Germany).

Effect of the extract on cells

HepG2 cells were grown at a density of 1 X 10^6/mL in DMEM in 96 well flat bottom culture plates and allowed to grow for 24 h before treatment with the extract. Cells were treated with different concentration of the extract starting from 0.15 mg/mL. After incubation for 24 h, the effect of the extract on the cell viability was assessed by MTT tetrazolium salt assay [15] by measuring the absorbance at 570 nm using ELISA Reader (Biorad, Model 680). Since the extract was dissolved in 1% DMSO, cells treated with 1% DMSO (in media) were taken as negative control while cells treated with 1% Triton X were used as positive control for cell death. All data on cell viability assay was taken as average of triplicate analyses and presented as mean±standard error (SE). Concentration of the extract which inhibits 50% growth of the cells (IC_{50}) was determined using the MTT assay. Percent viable cells were determined by dividing the optical density of treated cell by that of the vehicle-treated cells in the same 96-well plate. The concentration of the extract was then plotted against the percent cell survival. In this manner, a dose response curve was generated and the IC_{50} was determined. To determine the cytotoxic effect of the extract on normal cells, healthy lymphocytes cultured in RPMI were used.

Fluorescent staining of nuclei

HepG2 cells were grown on at a density of 1 X 10^6 cells / mL in petridishes containing cover slips for 24 h and then treated with the extract [16]. Nuclear morphology of both control and treated cells was assessed using DAPI. Briefly, 1 µL of 5 mg/mL DAPI was added in 1 mL of media containing the cells and incubated in dark at 37ºC for 10 min. At the end of incubation, the cells were washed with PBS and mounted on glass slides using aqueous mountant and nuclear morphology was observed using fluorescence microscope (E600, Nikon, Japan) at 330-380 nm. Same protocol was followed for propidium iodide (PI) to detect dead cells.

Results

Qualitative phytochemical analysis of the extracts

Phytochemical screening of *P. javanica* seeds extract revealed the presence of flavonoids, saponins, alkaloids, terpenoids, anthraquinones, steroids, reducing sugars and glycosides (Table 1). All the extracts showed positive result for alkaloids, flavonoids and anthraquinones. Terpenoids, steroids and saponins could not be detected in aqueous extract. Ethanolic extract showed negative result for reducing surgars and glycosides. Similarly, glycosides could not be detected in methanolic extract.

Table 1: Qualitative phytochemical analysis of different extracts of *P. javanica* seeds

Constituents	*Parkia javanica* seeds extract		
	Aqueous	Ethanolic	Methanolic
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	-	+	+
Glycosides	+	-	-
Reducing sugars	+	-	+
Steroids	-	+	+
Tannins	+	-	-
Terpenoids	-	+	+
Anthraquinones	+	+	+

Maximum absorbance (λmax) of the phytochemicals

The absorption spectra of *P. javanica* seeds extracts showed a number of peaks (Fig. 1). The aqueous extract showed a major peak at 366 nm and a very small peak at 354 nm. The ethanolic extract showed five peaks out of which the two major peaks were observed at 233 and 257 nm. The methanolic extract revealed more number of peaks at 222, 267, 307, 323, 341, 350 and 366 nm.

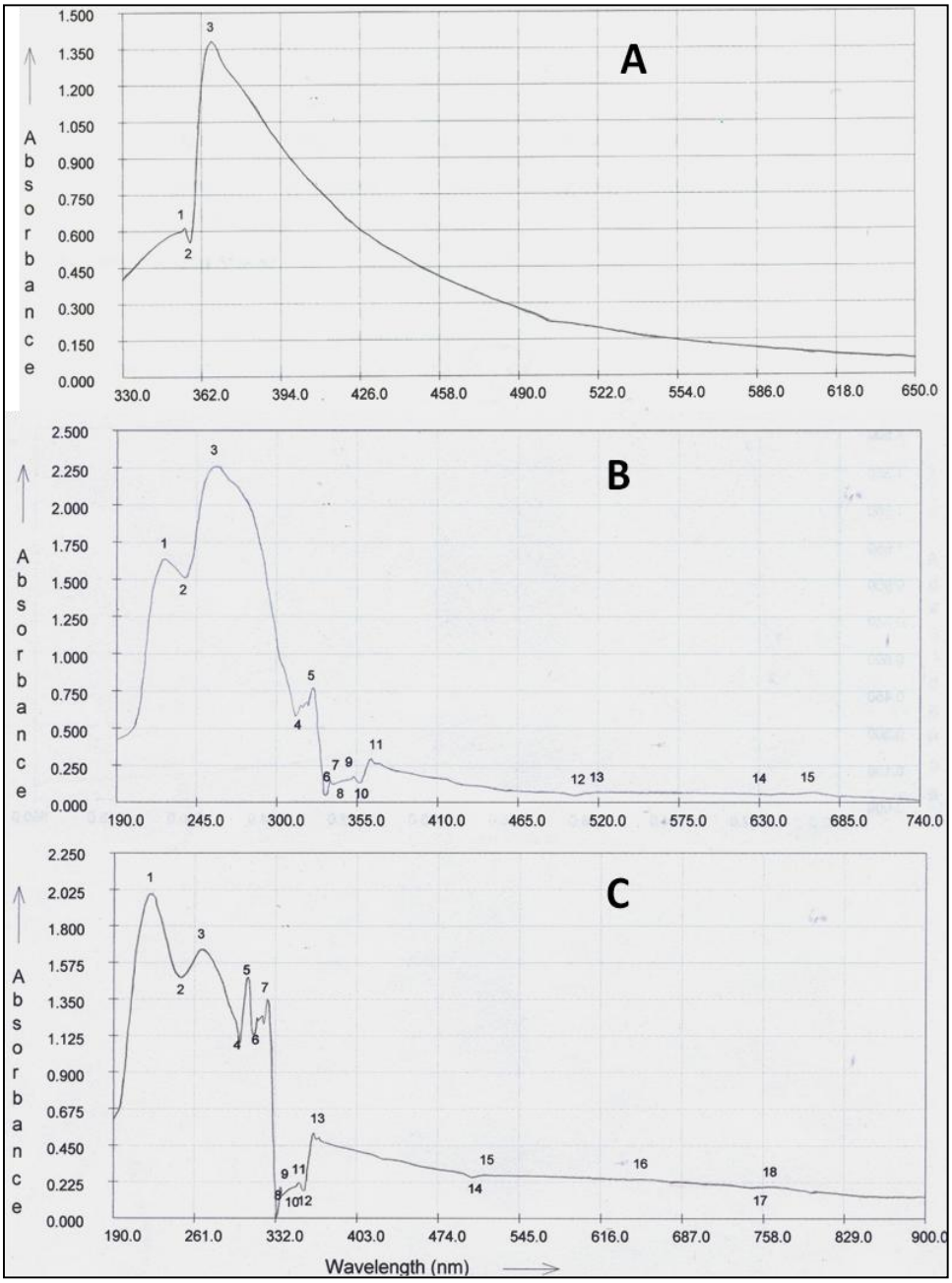

Fig 1: UV-Visual spectra of *P.javanica* seeds extracts (A) Aqueous (B) Ethanolic (C) Methanolic

Thin layer chromatography of the extracts

The TLC screening of the bioactive compounds covered flavonoids, phenolics, alkaloids, anthraquinones, terpenoids and saponins which are identified as spots on the TLC plates

(Fig. 2 and Fig. 3). The extracts showed one or more spots on the plates under different sovent systems except aqueous extract which developed no spots of phenolics and terpenoids. The retention factors (Rf) for each of the extracts in different solvent systems are detailed in Table 2.

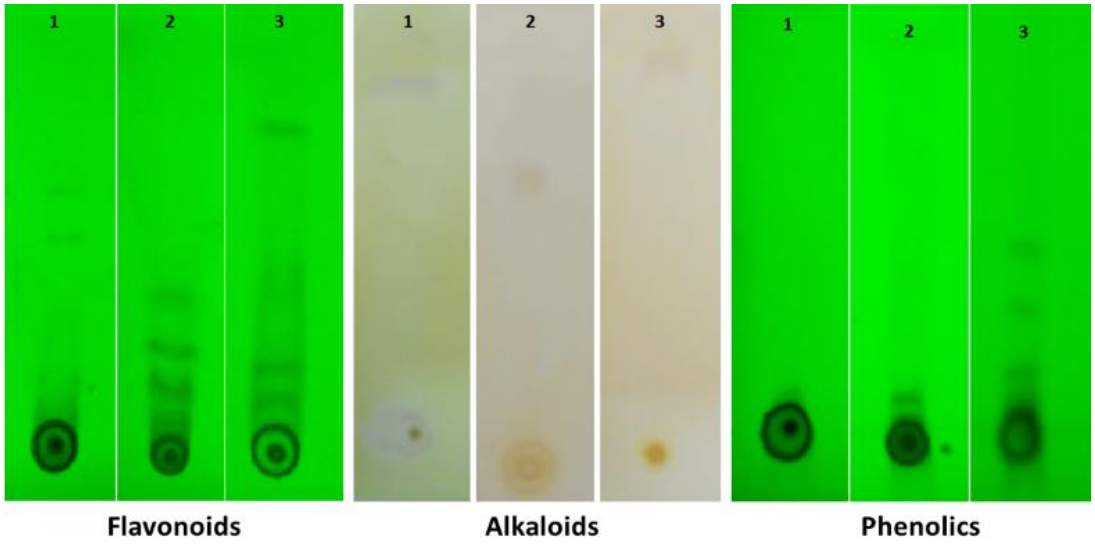

Fig 2: Thin Layer Chromatography for detection of Flavonoids, Alkaloids and Phenolics in the extracts 1: Aqueous extract 2: Ethanolic extract 3: Methanolic extract

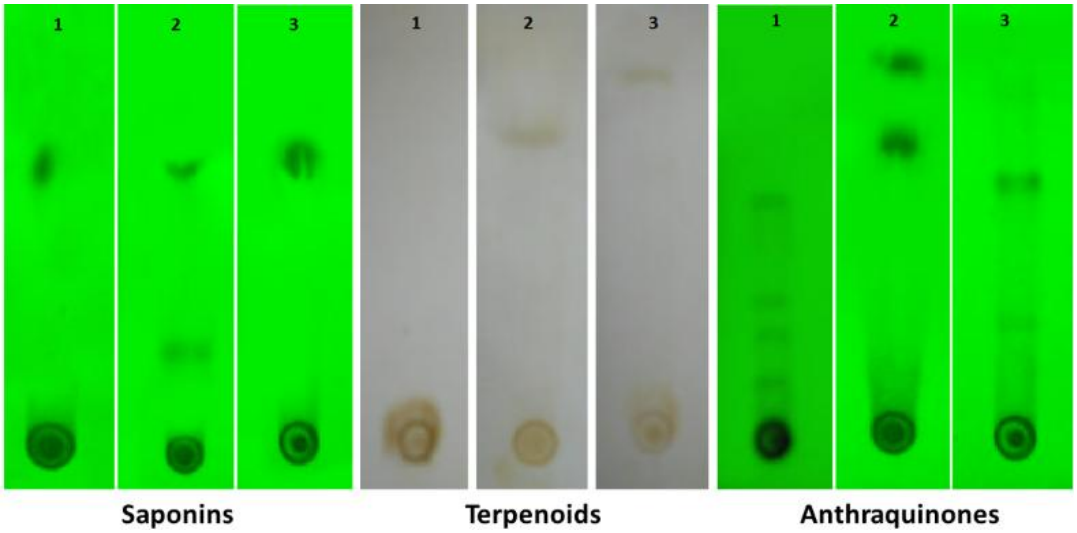

Fig 3: Thin Layer Chromatography for detection of Saponins, Terpenoids and Anthraquinones in the extracts 1: Aqueous extract 2: Ethanolic extract 3: Methanolic extract

Table 2: Thin Layer Chromatography for detection of phytochemicals present in the extracts of *P. javanica* seeds

Chemical name	Solvent systems	Types of extract	Rf values	Total bands	Spray reagent
Flavonoids	Chloroform : methanol (19:1)	Aqueous	0.57, 0.47	2	No reagent, UV light (254 nm)
		Ethanolic	0.32, 0.20, 0.13	3	
		Methanolic	0.14, 0.22, 0.75	3	
Phenolics	Chloroform : methanol (27:0.3)	Aqueous	-	0	Folin Ciocalteau's reagent
		Ethanolic	0.11	1	
		Methanolic	0.22, 0.34, 0.5	3	
Saponins	Chloroform : glacial aetic acid : methanol :water (64:34:12:8)	Aqueous	0.67	1	Iodine vapour
		Ethanolic	0.31, 0.62	2	
		Methanolic	0.65	1	
Anthraquinones	Ethyl acetate: toluene : acetic acid (8:4:1)	Aqueous	0.18, 0.31, 0.42, 0.60	4	5% methanolic KOH
		Ethanolic	0.71, 0.89	2	
		Methanolic	0.34, 0.64, 0.86	3	
Alkaloids	Methanol : conc. NH_4OH (200 :3)	Aqueous	0.84	1	Wagner's reagent
		Ethanolic	0.67	1	
		Methanolic	0.93	1	
Terpenoids	Ethyl acetate: toluene : acetic acid (8:4:1)	Aqueous	-	0	Anisaldehyde in H_2SO_4
		Ethanolic	0.83	1	
		Methanolic	0.88	1	

Cell viability and IC_{50}

Based on the phytochemical analysis, methanolic extract was selected for evaluation of anticancer effect against cancer cell. The extract was dissolved in 1% DMSO (in media) to a concentration of 5 mg/mL. The solution is then filtered using 0.2 µm syringe filter and used for cell culture investigations. The percent viable cells decreased with increased in the concentration of extract. HepG2 cells showed 4.59% viability when treated with 1 mg/mL of extract (Fig 4.). The effect of the extract on growth of normal healthy lymphocytes was also found to be dose dependent. IC_{50} of the extract calculated by plotting graph was found to be 1.41 mg/mL and 0.48 mg/mL respectively for lymphocytes and HepG2 cells.

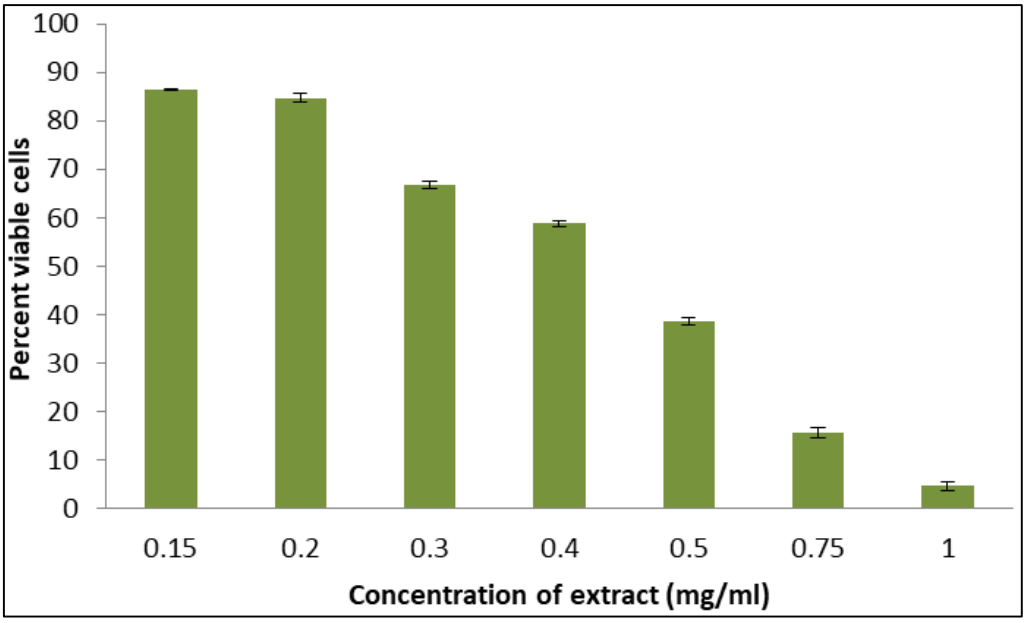

Fig 4: Percent of viable HepG2 cells at different concentrations of the extract. Values are expressed as mean±SE, n=3.

Fluorescent staining of the nuclei

HepG2 cells were treated with IC_{50} of the extract for 24 h and processed for observation of their nuclear morphology. HepG2 cells showed pyknotic nucleus but nuclear fragmentation was not very clear. Nucleus of the dead cells showed brighter colour as compared to control cells and are also positive for propidium iodide (Fig. 5).

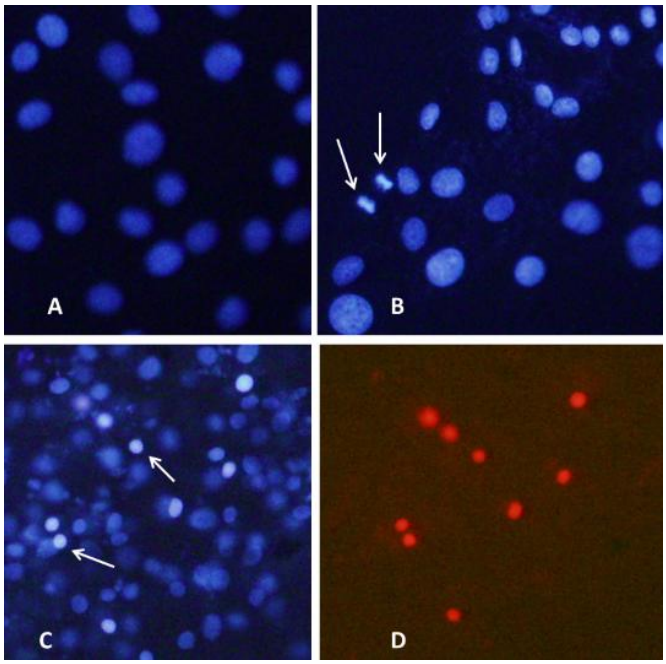

Fig 5: HepG2 cells A) Control treated with vehicle B) treated cells showing pyknotic nucleus C) light coloured nucleus of dead cells D) Dead cells of the same field (C) stained with PI.

Discussion

P. javanica is a popular tree among the people of north eastern states of India. It is known to the locals not only as a vegetable but as a traditional remedy for many diseases. In the present study, phytochemical analysis of *P. javanica* seeds extracts was carried out. In qualitative phytochemical analysis of aqueous, ethanolic and methanolic extracts of *P.javanica* seeds flavonoids could be detected. This is in contrast to the earlier findings on *Parkia speciosa* (another species under genus *Parkia*) where flavonoids could be detected in ethanolic extract but not in methanolic extract of the seeds [17, 18]. The extracts showed number of peaks at around 260 nm and 360 nm in UV-Vis spectrophotometry which is an indicative of the presence of flavonoids and other phenolic compounds [19-21]. Most phenolic compounds absorb at 260 nm while ellagic acid derivatives and flavonols have maximum absorption at 360 nm [19]. Presence of phenolic compounds in almost all parts of *Parkia speciosa* has been reported [22]. Similarly, in thin layer chromatography, spots of flavonoids and phenolics could be detected. In addition, terpenoids and saponins could also be detected in both ethanolic and methanolic extract. Terpenoid compounds, ß- sitosterol, squalene, stigmasterol, lupeol and campesterol were identified in *Parkia speciosa* seeds [23]. Presence of saponins, glycosides, alkaloids in other *Parkia* species had already been reported [24, 25].

Among the known phytochemicals, flavonoids are one of the most popular compounds with a variety of biological activities at nontoxic concentrations. Flavonoids have been widely discussed as promising anticancer agents [26, 27]. This group of compounds are also reported to produce a variety of effects against cancer cells such as inhibition of cell growth, induction of apoptosis, inhibition of kinase enzymes [28-30]. In our study, methanolic extract of *P. javanica* seeds was found to exhibit a dose dependent inhibitory effect on HepG2 cells

at a non-toxic concentration to normal cells. It is good that the effective concentration of the extract causes minimum cell death to normal lymphocytes because an ideal anticancer agent should be able to kill cancer cells without fatal toxicity on normal cells [31]. This anticancer or antiproliferative effect of the extract might have been the combined outcome of the phytochemicals present in the extract. Flavonoids have many effects on cancer cells including carcinogen inactivation, antiproliferation, cell cycle arrest, induction of apoptosis [32]. Terpenoids has numerous therapeutic properties including anticancer, antiparasitic, antiallergenic, anti-inflammatory and immuno-modulatory properties [33]. Likewise, the other compounds detected in the extracts, saponins, anthraquinones and alkaloids are also reported to have anticancer effect by various researchers. Growth inhibition and apoptosis induction properties of saponins in human colon cancer cells and tumor xenograft have been reported [34]. Anthraquinones are reported to inhibit the uptake of glucose in tumor cells, causing changes in membrane associated functions and led to cell death [35]. Many of the plant based anticancer drugs are alkaloids which can inhibit cell proliferation by preventing microtubule formation [36].

Fluorescent staining of nuclei with DAPI showed change in nuclear morphology of extract treated HepG2 cells. Changes in nucleus like chromatin condensation and nuclear fragmentation are the morphological hallmarks of apoptosis [37]. Clear nuclear fragmentation was not observed at 24 h though numerous dead cells with brighter coloured and PI positive nucleus were present. These observations are not specific to either apoptotic or necrotic type of cell death. It may also be possible that the treated apoptotic cells resulted in secondary necrotic death [38]. Though apoptosis had been observed in many cancer therapies, killing of cancer cells by direct toxicity had also been revealed [39]. In fact, phagocytosis of necrotic cancer cells may give rise to inflammatory pathways that can cause antitumor cytotoxicity [40]. Although the kind of cell death induced by the extract needs to be further investigated, it was very clear that methanolic extract of *P.javanica* seeds induce death in cancer cell at a non-toxic concentration to normal cells. Induction of cytotoxicity in *in-vitro* culture of sarcoma-180 (S-180), A549, AGS, and MDA-MB435 by aqueous methanolic extract of *P.javanica* seeds have been reported very recently [41]. As compared to their findings, the amount of extract to inhibit 50% cell viability in cancer cell was higher in our study which may be because of difference in the extraction protocol and types of cells used in the assay. Future studies may be targeted to optimise the extraction methods to obtain more bioactive compounds in minimum amount of extract and testing the extract against a wide range of cancer cells. Overall, the present study highlighted that *P.javanica* seeds are rich in phytochemicals which are beneficial for health and disease prevention and has the potential to inhibit the growth of cancer cell at a non-toxic concentration.

Conclusion

Phytochemicals present in the extracts of *P.javanica* seeds can well be correlated with their use in traditional medicine. The methanolic extract revealed the presence of alkaloids, flavonoids, saponins, terpenoids and anthraquinones which are known to possess chemopreventive effects. The extract was also found to inhibit proliferation of human liver cancer cell HepG2. Effect of the extract on cancer cells may vary depending on types of cells which can be determined by testing against different types of cancer cells. Other health beneficial effects of this plant need to be evaluated using suitable models. Additional studies may lead to discovery of bioactive compounds which may serve as a template for development of new affordable and safer drugs.

Acknowledgement

Dr. Chanu thanks Dr. Ayub Ali, Head, Department of Veterinary Biochemistry and Physiology, College of Veterinary Science and A.H. Selesih for extending the UV-Vis spectrophotometer facility for conducting the work. The authors are grateful to Director, IVRI for providing other necessary facilities and ICAR, New Delhi for financial assistance.

References

1. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. American Journal of Clinical Nutrition. 2003; 78:517S-20S.
2. Ningombam DS, Singh PK. Ethnobotanical Study of Phologacanthus thyrsiformis Nees: A Conserved Medicinal Plant of Manipur, Northeast India. International Journal of Herbal Medicine. 2014; 1(5):10-14.
3. Mao AA, Hynniewta TM, Sanjappa M. Plant wealth of Northeast India with reference to ethnobotany. Indian Journal of Traditional Knowledge. 2009; 8(1):96-103.
4. Rathi RS, Misra AK, Roy S, Verma SK, Singh SK. Potential of a lesser known tree species *Parkia roxburghii* G. Don of North East India. Indian Forester. 2012; 138(5):476-479.
5. Suvachittanont W, Kurashima Y, Esumi H, Tsuda M. Formation of thiazolidine-4-carboxyiic acid (thioproline), an effective nitrite-trapping agent in human body, in *Parkia speciosa* seeds and other edible leguminous seeds in Thailand. Food chemistry. 1996; 55(4):359-363.
6. Tahira T, Tsuda M, Wakabayashi K, Nagao M, Sugimura T. Kinectics of nitrosation of thioproline, the precursor of a major nitroso compound in human urine, and its role as a nitrite scavenger. Gann; the Japanese journal of cancer research. 1984; 75(10):889-94.
7. Tahira T, Ohgaki H, Wakabayashi K, Nagao M, Sugimura T. The inhibitory effect of thioproline on carcinogenesis induced by N-benzylmethylamine and nitrite. Food and Chemical Toxicology. 1988; 26(6):511-6.
8. Kaur N, Singh J, Kamboj SS, Agrewala JN, Kaur M. Two novel lectins from *Parkia biglandulosa* and Parkia roxburghii: isolation, physiochemical characterization, mitogenicity and anti-proliferative activity. Protein and Peptide Letters. 2005; 12(6):585-95.
9. La Vecchia C, Altieri A, Tavani A. Vegetables, fruit, antioxidants and cancer: a review of Italian studies. European Journal of Nutrition. 2001; 40:261-267.
10. Chanu KV, Ali A, Kataria M. Antioxidant Activities of Two Medicinal Vegetables: *Parkia javanica* and *Phlogacanthus thyrsiflorus*. International Journal of Pharmacy and Pharmaceutical Sciences. 2012; 4(1):102-106.
11. Peach K, Tracey MV. Modern Methods of Plant Analysis. Springer Verlag, Berlin, 1956, 1.
12. Radeleff RD. Veterinary Toxicology. 2nd ed. Lea and Febiger, Philadelphia, 1970.

13. Wahab OM, Ayodele AE, Moody JO. TLC phytochemical screening in some Nigerian Loranthaceae. Journal of Pharmacognosy and Phytotherapy. 2010; 2(5):64-70.
14. Mungole AJ, Awati R, Chaturvedi A, Zanwar P. Preliminary phytochemical screeining of *Ipomea obscura* (L)-A hepatoprotective medicinal plant. International Journal Pharm Tech Research. 2010; 2(4):2307-2312.
15. Hansen MB, Nielsen SE, Berg K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. Journal of Immunological Methods. 1989; 119:203-210.
16. Kirana C, Record IR, McIntosh GH, Jones GP. Screening for antitumor activity of 11 species of indonesian zingiberaceae using human MCF-7 and HT-29 cancer cells. Pharmaceutical Biology. 2003; 41(4):271-276.
17. Maisuthisakul P, Pasuk S, Ritthiruangdej P. Relationship between antioxidant properties and chemical composition of some Thai plants. Journal of Food Composition and Analysis. 2008; 21(3):229-240.
18. Miean KH, Mohamed S. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. Journal of Agricultural and Food Chemistry. 2001; 49(6):3106-3112.
19. Aaby K, Ekeberg D, Skrede AG. Characterization of phenolic compounds in strawberry (Fragaria X ananassa) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity. Journal of Agricultural and Food Chemistry. 2007; 55:4395-4406.
20. Dugo P, Donato P, Francesco C, Germano MP, Rapisarda A, Mondello L. Characterization of the polyphenolic fraction of *Morus alba* leaves extracts by HPLC coupled to a hybrid IT-TOF MS system. Journal of Separation Science. 2009; 32:3627-3634.
21. Lin LZ, Chen P, Oxcan M, Harnly JM. Chromatographic profiles and identification of new phenolic components of *Gingko biloba* leaves and selected products. Journal of Agricultural and Food Chemistry. 2008; 56:6671-6679.
22. Kamisah Y, Othman F, Qodriyah HMS, Jaarin K. *Parkia speciosa* Hassk.: A potential phytomedicine. Evidence-Based Complementary and Alternative Medicine. 2013; 709028.
23. Salman Z, Mohd Azizi CY, Nik Norulaini NA, Mohd Omar AK, Gas chromatography/Time-of-Flight mass spectrometry for identification of compounds from *Parkia speciosa* seeds extracted by supercritical carbon dioxide. Proceedings of the 1st International Conference on Natural Resources Engineering & Technology, Putrajaya, Malaysia, 2006, 112-120.
24. Komolafe K, Oyelade WA. Phytochemical Screening and *in Vitro* Antioxidant Activity of *Parkia biglobosa* Extract. Journal of Biology, Agriculture and Healthcare. 2015; 5(9):91-95.
25. Balaji K, Nendumaran SA, Devi T, Sikarwar MS, Fuloria S. Phytochemical analysis and in vitro antioxidant activity of *Parkia speciosa*. International Journal of Green Pharmacy. 2015; 9(4):S50-S54.
26. Ren W, Qiao Z, Wang H, Zhu L, Zhang L. Flavonoids: promising anticancer agents. Medicinal Research Reviews. 2003; 23(4):519-534.
27. Aggarwal BB, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. Biochemical Pharmacology. 2006; 71:1397-1421.
28. Weng MS, Liao CH, Chen CN, Wu CL, Lin JK. Propolin H from Taiwanese propolis induces G1 arrest in human lung carcinoma cells. Journal of Agricultural and Food Chemistry. 2007; 55:5289-5598.
29. Lee JC, Lee KY, Son YO, Choi KC, Kim SW, Hwang HJ *et al*. Plant originated glycoprotein, G-120, inhibits the growth of MCF-7 cells and induces their apoptosis. Food and Chemical Toxicology. 2005; 43:961-968.
30. Yagura T, Motomiya T, Ito M, Honda G, Iida A, Kiuchi F *et al*. Anticarcinogenic compounds in the Uzbek medicinal plant, *Helichrysum maracandicum*. Nature Medicine. 2008; 62:174-178.
31. Das A. Anticancer Effect of Anti-Malarial Artemisinin Compounds. Annals of Medical and Health Science Research. 2015; 5(2):93-102.
32. Chahar MK, Sharma N, Dobhal MP, Joshi YC. Flavonoids: A versatile source of anticancer drugs. Pharmacognosy Reviews. 2011; 5(9):1-12.
33. Ajikumar PK, Tyo K, Carlsen S, Mucha O, Phon TH, Stephanopoulos G. Terpenoids: Opportunities for biosynthesis of natural product drugs using engineered microorganisms. Molecular Pharmaceutics. 2008; 5(2):167-190.
34. Tin MM, Cho CH, Chan K, James AE, Ko JK. *Astragalus* saponins induce growth inhibition and apoptosis in human colon cancer cells and tumour xenograft. Carcinogenesis. 2007; 28:1347-55.
35. Huang Q, Lu G, Shen HM, Chung MC, Ong CN, Anticancer properties of anthraquinones from rhubarb. Medicinal Research Reviews. 2007; 27:609:630.
36. Beck WT, Cass CE, Houghton PJ. Microtubule targeting anticancer drugs derived from Plants and Microbes: Vinca alkaloids, Taxanes, and Epothilones. Edn 5, Hamilton (ON), BC Decker, 2000.
37. Ziegler U, Groscurth P. Morphological features of cell death. News in Physiological Sciences. 2004; 19:124-128.
38. Sawai H, Domae N. Discrimination between primary necrosis and apoptosis by necrostatin-1 in Annexin V-positive/propidium iodide-negative cells. Biochemical and Biophysical Research Communications. 2011; 411(3):569-73.
39. Gerl R, Vaux DL. Apoptosis in the development and treatment of cancer. Carcinogenesis. 2005; 26(2):263-270.
40. Fronseca C, Dranoff G. Capitalizing on the immunogenicity of dying tumor cells. Clinical Cancer Research. 2008; 14(6):1603-8.
41. Patra K, Jana S, Sarkar A, Karmakar S, Jana J, Gupta M *et al*. *Parkia javanica* Extract Induces Apoptosis in S-180 Cells via the Intrinsic Pathway of Apoptosis. Nutrition and Cancer. 2016, 1-19.