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).

© 2018 TPI 2018; 7(311): 305-311
All the plastic wares including microfuge tubes, micropipettes, and glass slides 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 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:

\[ Rf = \frac{\text{Distance moved by the compound}}{\text{Distance moved by the solvent front}} \]

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% CO2 at 37°C in a CO2 incubator (Binder, Germany).

Effect of the extract on cells

HepG2 cells were grown on at a density of 1 X 106/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 (IC50) 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 IC50 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 106 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 37°C 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 sugars and glycosides. Similarly, glycosides could not be detected in methanolic extract.
Table 1: Qualitative phytochemical analysis of different extracts of *P. javanica* seeds

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Parkia javanica seeds extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
</tr>
</tbody>
</table>

Maximum absorbance (\(\lambda_{max}\)) 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 solvent 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](image)

![Fig 3: Thin Layer Chromatography for detection of Saponins, Terpenoids and Anthraquinones in the extracts 1: Aqueous extract 2: Ethanolic extract 3: Methanolic extract](image)

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

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Solvent systems</th>
<th>Types of extract</th>
<th>Rf values</th>
<th>Total bands</th>
<th>Spray reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>Chloroform : methanol (19:1)</td>
<td>Aqueous</td>
<td>0.57, 0.47</td>
<td>2</td>
<td>No reagent, UV light (254 nm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanolic</td>
<td>0.32, 0.20, 0.13</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Phenolics</td>
<td>Chloroform : methanol (27:0.3)</td>
<td>Aqueous</td>
<td>-</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanolic</td>
<td>0.11</td>
<td>1</td>
<td>Folin Ciocalteu’s reagent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanolic</td>
<td>0.22, 0.34, 0.5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Saponins</td>
<td>Chloroform : glacial aetic acid : methanol :water (64:34:12:8)</td>
<td>Aqueous</td>
<td>0.67</td>
<td>1</td>
<td>Iodine vapour</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanolic</td>
<td>0.31, 0.62</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanolic</td>
<td>0.65</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Ethyl acetate: toluene : acetic acid (8:4:1)</td>
<td>Aqueous</td>
<td>0.18, 0.31, 0.42, 0.60</td>
<td>4</td>
<td>5% methanolic KOH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanolic</td>
<td>0.71, 0.89</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanolic</td>
<td>0.34, 0.64, 0.86</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Methanol : conc. NH$_4$OH (200 :3)</td>
<td>Aqueous</td>
<td>0.84</td>
<td>1</td>
<td>Wagner’s reagent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanolic</td>
<td>0.67</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanolic</td>
<td>0.93</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Ethyl acetate: toluene : acetic acid (8:4:1)</td>
<td>Aqueous</td>
<td>-</td>
<td>0</td>
<td>Anisaldehyde in H$_2$SO$_4$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanolic</td>
<td>0.83</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanolic</td>
<td>0.88</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
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.

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).

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.

![Fig 4: Percent of viable HepG2 cells at different concentrations of the extract. Values are expressed as mean±SE, n=3.](image-url)
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 have 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-MB-435 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. Seleshi 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**


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.


