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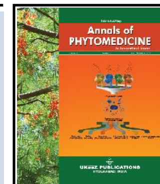

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Qualitative and quantitative phytochemical screening and *in vitro* cytotoxicity study of *Zanthoxylum armatum* DC. and *Pleurospermum angelicoides* (DC.) Benth. ex C.B. Clarke : Important medicinal plants of the upper Himalayan region

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Abstract

Medicinal plants are gaining immense importance due to the therapeutic potential of various phytocompounds present in them. These compounds are phytochemicals like alkaloids, flavonoids, phenols, terpenes, saponins, lignins, phytosterols, tannins, aldehydes, proteins, fatty acids, glycosides, coumarins, *etc*. The present investigation aimed to screen the *Zanthoxylum armatum* DC. and Pleurospermum angelicoides (DC.) Benth. ex C.B. Clarke for the qualitative and quantitative presence of different phytochemicals in them. Aqueous and ethanolic extracts of *Z. armatum* (fruit kernel and seed) and *P. angelicoides* (roots) were screened using qualitative tests for flavonoids, alkaloids, proteins, aldehyde, phytosterols, phenols along with quantitative assays for total phenolic and flavonoids content, ferric reducing antioxidant power assay (FRAP) and ascorbate - iron (III) - catalyzed phospholipid peroxidation (AICPP) activity. An aqueous extract of *Z. armatum* (fruit kernel) revealed a stronger reaction for the presence of flavonoids, tannins, saponins and phytosterols. Alkaloids and aldehydes were reported to be present in seeds of *Z. armatum*. *P. angelicoides* observed strong reactions for alkaloids and saponin's presence. Total phenol and flavonoid content of aqueous and ethanolic extracts of *Z. armatum* (fruit kernel) were 33.24 ± 1.98 TPC (mg GAE/g extract), 33.09 ±.35 TPC (mg GAE/g extract), 5.43 ± 0.46 TFC (mg CE/g extract) and 4.47 ± 0.77 TFC (mg CE/g extract), respectively. Per cent inhibition activity of AICPP was reported to be 66.97 ± 5.76 and 45.71 ± 10.53, per cent in both the extracts of fruit kernel of *Z. armatum* whereas, 37.27 ± 4.34 and 67.35 ± 4.47 per cent inhibition activities were reported in both extracts of *P. angelicoides,* respectively. Highest FRAP activity of 0.362 ± 0.01 (mmol Fe^{2+}/gm extract), followed by 0.116 ± 0.005 (mmol Fe^{2+}/gm extract) was reported in aqueous and ethanolic extracts of *Z. armatum*. The ethanolic fraction of *P. angelicoides* was found safer among all the extracts in the cytotoxicity study.

1. Introduction

The Indian Himalaya region (IHR) is a home tract for more than 8000 vascular plants (Singh and Hajra, 1996), out of these, 1748 are reported to have known medicinal values (Samant *et al*., 1998). The Himalayan region is the hotspot of various medicinal or herbal plants which serves the medicinal and food requirements of local tribal inhabitants of the Himalayas. Traditional medicines are used above 60 per cent population of the world (Ballabh and Chaurasia, 2007). Medicinal plants are gaining importance for the treatment and management of various diseases in humans and animals due to their low cost, ease of availability and lesser side effects. Phytochemicals are secondary plant metabolites that are biologically active and provide health benefits (Hasler and Blumberg, 1999). They are protecting the plants either from disease or external traumas. Phytochemicals are responsible for color, flavor and aroma development in plants. These secondary plant metabolites in general give protection against physical hazards like-UV exposure, insect attack, pollutants, biotic or a biotic stresses and drought conditions (Mathai, 2000 ; Gibson *et al*., 1998). Besides these, phytochemicals have immense importance in the treatment of various chronic diseases like cancer and liver and heart diseases. Phytochemicals have multiple therapeutic uses in humans and animals, against various disease conditions. *Z. armatum* is an evergreen, tiny, sub-deciduous, and spiny tree, commonly found in the valleys of the Himalayas, the north-east part of India, Nepal, Bhutan, Pakistan, Myanmar, and Bangladesh. The vernacular names of the plant are: Indian prickly ash, Winged prickly ash, Timur and Timru. This plant is being used by local herbal therapists against several diseases without having any side effects. The fruits and seeds of the Timur are being used as remedies against fever, dyspepsia and parasitic diseases (Kalia *et al*., 1999). *P. angelicoides* (Local name: Gandrani, Gandrayani, Chipi) is an important medicinal plant of the upper Himalayan region. It belongs to the family Apiaceae. Roots of Gandrani are being used as flavoring agent during food preparation in the Uttarakhand region. Decoction of root, along with cumin,

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and black pepper are reported to have activity against pyrexia and chronic gastric disorders (Phondani *et al*., 2010; Nautiyal *et al*., 2001). Herbal remedies are a complex mixture of different species of medicinal plants which contains various phytocompounds in different concentrations. It is important to have quality standards for herbal products with safe preclinical and clinical studies. To elucidate the chemicals present in medicinal plants, important analytical techniques are being employed like thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) along with capillary electrophoresis technique. Advanced analytical techniques based on metabolic fingerprinting, infrared spectroscopy and quantitative nuclear magnetic resonance spectra are also effective for the chemoprofiling of plants (Efferth and Greten, 2012). In recent world, the role of free radicals got an increased attention due to its involvement in disease biology (Taha *et al.,* 2019). Therefore, the herbal medicinal plants with potent antioxidant activity will become a hot topic of research in coming future. The preset study aimed to investigate the qualitative and quantitative phytochemical analysis and *in vitro* cytotoxicity assay of two important medicinal plants of the Himalayan region, *viz*., *P. angelicoides* and *Z. armatum*.

2. Materials and Methods

2.1 Collection of plant material and extraction

Zanthoxylum armatum DC. (Common names:Timur, Timru) and *Pleurospermum angelicoides* (DC.) Benth. ex C.B. Clarke (Common names: Gandrani, Chipi), were collected from the Munsiyari region of the Pithoragarh district of Uttarakhand. The plant materials were authenticated by the Dr. Nidhi Sharma, Scientist (Forestry and Agroforestry) at ICAR-IVRI, Mukteswar, Nainital, Uttarakhand. The specimens are maintained at Institute with Herbarium No.ZAF-20-/IVRI, 2020-21 and PACR-03/IVRI, 2020-21. The *Z. armatum* (fruit kernel, and seeds) and *P. angelicoides* (roots) were cleaned and air dried and pulverized in the electric grinder. These plant materials (15-20 gm in thimble) were subjected to extraction using 200 ml water or absolute ethanol as a solvents, using columnar Soxhlet method at a temperature of 40-41°C for 4-6 h duration. The per cent yield of aqueous and ethanolic extracts was calculated. The extracts were dried at 41°C temperature for further analysis.

2.2 Phytochemical analysis

A total of six different extracts of Timur (fruit kernel and seeds) and Gandrani (roots) were prepared. After extraction, the extracts were vacuum-evaporated for further analysis.

2.2.1 Qualitative phytochemical analysis

The qualitative phytochemical analysis was carried out to detect basic compounds like alkaloids, saponins, phenols, flavonoids and tannins in different extracts. The following tests were carried out per standard methods (Ansari *et al.,* 2020; Dubey *et al.,* 2020; Tiwary *et al*., 2011).

2.2.1.1 Test for alkaloids

The extracts were dissolved in N/10 hydrochloric acid and filtered through Whatman filter paper for the further test procedure.

a. **Mayer's test:** The acidic filtrates from above were mixed with freshly prepared Mayer's reagent (Mix 1.36 g mercuric chloride and 5.0 g potassium iodide in 100 ml distilled water) slowly. The precipitate of yellow color indicates the presence of alkaloids in tested extracts.

b. **Wagner's test**: Acidic filtrates of extracts were slowly mixed with Wagner's reagent (Mix 2.0 g iodine and 6.0 g potassium iodide in 100 ml distilled water). It will form a brown/reddish precipitate which indicates the presence of alkaloids in the tested substance.

2.2.1.2 Test for flavonoids

a. **Alkaline reagent test:** Extracts solutions were mixed with a few drops of alkaline sodium hydroxide solution. It developed a intense yellow color which got decolorized after the addition of N/10 HCl.

b. **Lead acetate test:** 10 % lead acetate solution was mixed with extract solutions. The formation of yellow color indicates the qualitative presence of flavonoids in tested samples.

2.2.1.3 Test for phenols

a. **Ferric chloride test:** The test extracts were mixed with a 8-10 drops of ferric chloride solution. The appearance of dark bluish-black color indicates the presence of phenols in the tested substance.

b. **Gelatin test:** To the test solutions, 1% gelatine solution containing sodium chloride was mixed. The presence of phenolic compounds will make white precipitation.

2.2.1.4. Test for saponins

a. **Foam test:** Approximately 0.5 g of the crude extract was mixed with 2.0 ml of water and vigorously shaken for a while. The persistence of foam formation more than ten minutes indicates the presence of saponins in the tested solutions.

2.2.1.5. Test for phytosterols

a. **Salkowski's test:** The test extracts solutions were treated with chloroform and filtered through Whatman filter paper. Filtrates were treated few drops of concentrated sulphuric acid. The solution was mixed well and allowed to stand for some time. The presence of golden yellow color in the test solution indicates the presence of triterpene compounds.

2.2.1.6. Test for protein

a. **Xanthoproteic test:** Add few drops of concentrated nitric acid to the extracts solutions. Presence of yellow gives an indication for the presence of proteins in tested samples.

b. **Biuret test:** To the test solutions, add 4% sodium hydroxide solution and few drops of 1% copper sulphate solution. Violet colour appearance indicates for protein presence.

2.2.1.7 Test for aldehydes

a. **Schiff's test:** To the test solutions, add few drops of Schiff's reagent. The magenta colour development gives an indication for aldehyde presence.

2.2.2 Quantitative phytochemical analysis

The following *in vitro* tests were conducted to assess the antioxidant activity of the extracts.

2.2.2.1 Ferric reducing antioxidant power assay (FRAP)

This assay determines the ability of the samples to reduce ferric (III) iron to ferrous (II) iron. The assay was carried out according to the protocol of Sahgal *et al*. (2009). FRAP reagent of the assay was prepared using acetate buffer (25 ml, 300 mmol/l, pH 3.6), 10 mmol/l TPTZ solution (2.5 ml) in 40 mmol/l HCl and 20 mmol/l $FeCl_3$ solution (2.5 ml) in 10:1:1 (v/v) proportions accordingly. The reagent was fresh and warmed to 37^0C before its use. Different extract samples (150 ml volume) were mixed with the FRAP reagent (4.5 ml). The optical density of the reaction mixture was taken at 593 nm after 4 min time period. The samples were analyzed in triplicates for more accuracy. The standard curve of the $FeSO_4$ solution was constructed using different concentrations (0.1-1.0 mg/ml). The results were expressed as mmol Fe (II) per gram dry weight of plant extracts. Vitamin C was kept as a comparative model in this assay.

2.2.2.2 Ascorbate - iron (III) - catalyzed phospholipid peroxidation (AICPP)

The assay measures the ability of the extracts to scavenge the hydroxyl radicals by the modified method of Aruoma *et al.* (1997). Fresh goat liver tissue was mixed (1:10) with 10 mM phosphate-buffered saline (PBS, pH 7.4) and sonicated in an ice bath for preparation of the homogenate liposomes. The liposomes (0.2 ml) solution was added with 0.5 ml of PBS buffer, 0.1 ml of 1 mM $FeCl_3$, and various volumes (100 µl and 200 µl) of plant extracts after that 0.1 ml volume of 1 mM vitamin C was added to it. After incubation at 37^0C for 60 min, 1 ml of 10% trichloroacetic acid (TCA) was added and centrifuged at 2000 rpm for 10 min at room temperature. At final, 1 ml of 0.67% 2-thiobarbituric acid (TBA) in 0.05 M NaOH was added to the supernatant, vortexed and heated in a water bath at 100^0C for 20 min. The solution was kept for cooling and then 1 ml of distilled water was added and absorbance was recorded at 532 nm. The control solution contained all reagents except the extract samples. The assay runs with triplicate samples for accuracy. Vitamin E was used as standard.

The percentage (%) inhibition activity was calculated using the formula below:

[(Abs. of control – Abs. of the sample)/Abs. of control] × 100

2.2.2.3 Total flavonoids content (TFC)

The total flavonoid content of the extracts was determined using the colorimetric method as described by Nabavi *et al.* (2008). The extract sample solution (0.5 ml) was mixed with distilled water (2 ml) and 0.15 ml of 5% sodium nitrate ($NaNO_2$) solution. After 6 min of the incubation period, 0.15 ml volume of 10% aluminium chloride ($AlCl_3$) solution was added and then allowed to stand for 6 min, followed by the addition of 4% NaOH solution (2 ml) to the mixture. Consequently, distilled water was added to the sample to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for 15 min time. The mixture's optical density was determined at a wavelength of 510 nm. The total flavonoid content was expressed in mg of catechin equivalent (CE) per gram of extract sample.

2.2.2.4 Total phenolic content (TPC)

The total phenolic content of the extracts was measured using the Folin-Ciocalteu method (Biglari *et al*., 2008). The samples (0.4 ml; 1mg/ml concentration) were taken into the test tubes. Distilled water (1.0 ml) and Folin-Ciocalteu reagent (1.0 ml) were added to the sample solution and mixed well. After 1 min, sodium carbonate solution (Na_2CO_3, 1.6 ml, 7.5%) was added and the mixture was allowed to stand for 30 min with intermittent shaking. A linear dose-response regression curve was generated using different concentrations of gallic acid and its absorbance at 765 nm wavelength. The TPC concentrations in the extracts were expressed as milligrams of gallic acid equivalent per gram of dry weight of extract (mg GAE/ g). All the methods were performed as per standard protocols.

2.3 *In vitro* cytotoxicity assay

In vitro cytotoxicity assay of *Z. armatum* and *P. angelicoides* (aqueous and ethanolic extracts) was done in MDBK cell lines as per OECD guidelines. The test concentrations used were from 1000 µg/ml,100 µg/ml, 10 µg/ml, 0.1 µg/ml, 0.01 µg/ml, 0.001 µg/ml, 0.0001 µg/ml, respectively. TD_{50} concentration (µg/ml) and maximum non toxic concentration (µg/ml) was calculated.

2.4 Statistical analysis

Data was subjected for statistical analysis using ANOVA wherever necessary and $p<0.05$ was considered statistically significant (Snedecor and Cochran, 1994).

3. Results

3.1 Extraction procedure and yield

Aqueous and ethanolic extracts of *Z. armatum* [fruit and its kernel: Figure 1(a)] and *P. angelicoides* [root: Figure1(b)] were prepared by the Soxhlet extraction method at 41^0C. The per cent yield of aqueous and ethanolic extracts of *Z. armatum* (fruit) and *P. angelicoides* (root) were 22.30; 30.0; 21.42 and 28.30 per cent, respectively, whereas aqueous and ethanolic extracts of seeds of *Z. armatum* showed 10.71 and 17.85 per cent yield only.

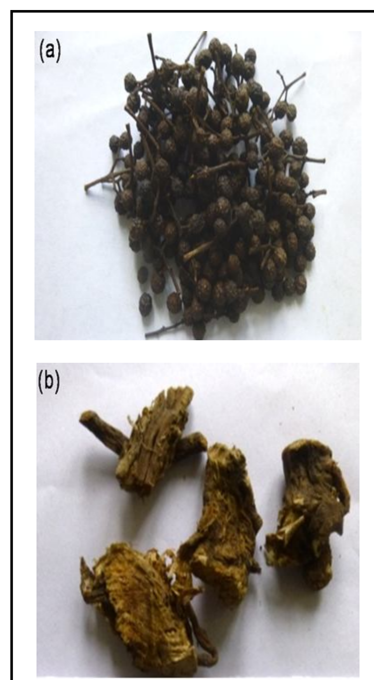

Figure 1: **(a) *Z. armatum* (fruits) and (b) *P. angelicoides* (roots).**

Table 1: Qualitative phytochemical analysis of different extracts [Aq- aqueous; Eth- ethanolic; (+++) strong; (++) moderate; (+) mild, and (–) negative]

Extracts	Flavanoids		Phenols		Alkaloids		Saponin	Phytosterol	Protein		Aldehyde
	Alkaline reagent test	Lead acetate test	$Fecl_3$ test	Gelatin test	Wagner test	Mayer's test	Foam test	Salkowski test	Xantho-proteic test	Biuret test	Schiff's test
Timur fruit kernel (Aq)	+++	+++	+++	+	++	-	+++	+++	+	++	+
Timur fruit kernel (Eth)	++	++	+	-	+	-	++	+++	-	-	-
Timur fruit seed (Aq)	+	+	++	-	++	+++	++	++	+	+	-
Timur fruit seed (Eth)	-	-	-	-	++	++	-	+	_	-	+++
Gandrani (Aq)	-	+	++	-	+++	+++	++	++	-	-	-
Gandrani (Eth)	-	+	++	-	++	++	+++	+	-	+	-

3.2 Qualitative phytochemical analysis

The qualitative phytochemical analysis of different plant extracts are summarized in Table 1 and Figure 2. An aqueous extract of fruit kernel of *Z. armatum* revealed a strong positive reaction for flavonoids, tannins, saponins and phytosterols whereas, a weak positive reaction was observed for alkaloids, proteins and aldehydes and weak reactions for flavonoids, phenols and alkaloids. An ethanolic extract of fruit kernel of *Z. armatum* revealed a strong reaction for phytosterols. Timur seed aqueous extract recorded the strong presence of alkaloids with weak reactions for phenols, saponins, phytosterols and proteins. Ethanolic extract of seed of *Z. armatum* showed a strong positive reaction for aldehydes only. An aqueous extract of *P. angelicoides* observed a strong positive reaction for alkaloids with weak reactions for phenols, saponins, and phytosterols. Ethanolic extract of *P. angelicoides* revealed a strong reaction for saponins with weak reactions for alkaloids and phenols.

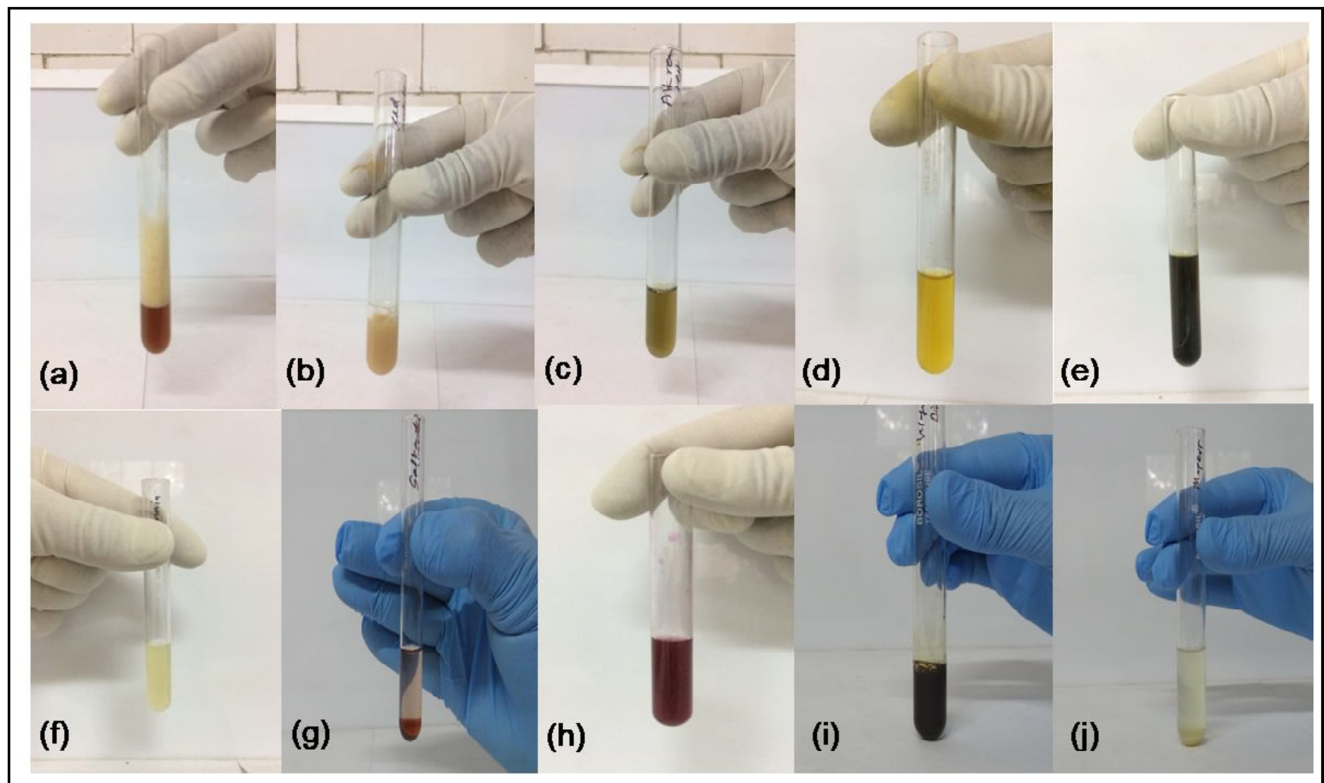

Figure 2: Qualitative phytochemical analysis revealing (a) Foam test, (b) Lead acetate test, (c) Alkaline reagent test, (d) Xanthoproteic test, (e) $FeCl_3$ test, (f) Gelatin test, (g) Salkowski test, (h) Schiff's test, (i) Wagner test, and (j) Mayer's test.

3.3 Quantitative phytochemical analysis

The total phenol and flavonoid content of all extracts are shown in Table 2. Linear regression curves for calculations of total phenolic and flavonoid activity are shown in Figures 3 and 4, respectively. TPC and TFC contents of aqueous extract of *Z. armatum* (fruit kernel) were 33.24 ± 1.98 mg GAE/g extract and 5.43 ± 0.46 mg CE/g extract, respectively. Ethanolic extracts of *Z. armatum* (fruit kernel) revealed the 33.09 ±.35 mg GAE/g extract TPC and 4.47 ± 0.77 mg CE/g extract TFC. The TPC of the aqueous extract of *P. angelicoides* was evidenced as 14.70 ± 0.36 mg GAE/g extract.

Table 2: Total phenolic and flavonoid content of the extracts (Aq-aqueous; Eth-ethanolic)

Extracts	Timur kernel (Aq)	Timur kernel (Eth)	Timur seed (Aq)	Timur seed (Eth)	Gandrani (Aq)	Gandrani (Eth)
TPC (mg GAE/gm extract)	33.24 ± 1.98[d]	33.09 ± 0.35[d]	19.99 ± 0.62 [c]	6.19 ± 1.55[a]	14.70 ± 0.36 [b]	7.48 ± 0.65[a]
TFC (mg CE/gm extract)	5.43 ± 0.46[a]	4.47 ± 0.77[a]	-	1.75 ± 0.72[c]	-	-

Data are expressed as mean ± SE. Different superscripts in the same row vary significantly (p<0.05).

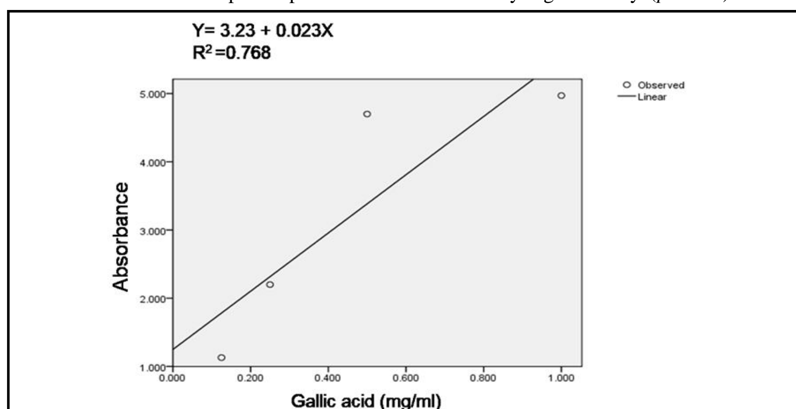

Figure 3: Linear regression curve for the calculation of total phenolic content.

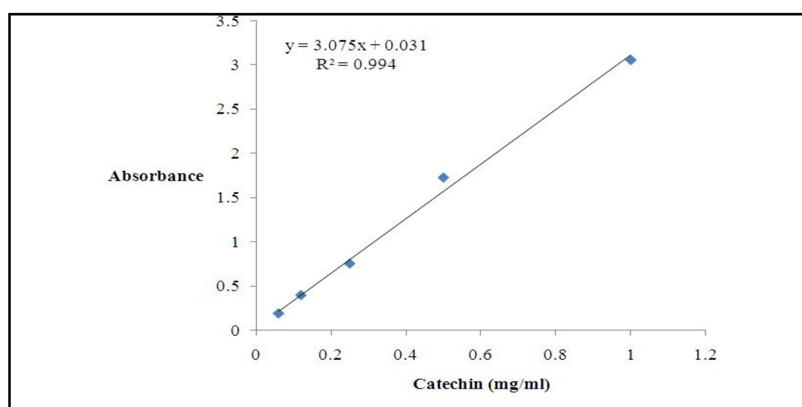

Figure 4: Linear regression curve for the calculation total flavanoids (510 nm).

Results of the percent AICPP and FRAP activities of different extracts are given in Table 3. Linear regression curve for calculation of FRAP activity is shown in Figure 5. Per cent inhibition activity of AICPP was reported to be 66.97 ± 5.76 and 45.71 ± 10.53, per cent in aqueous and ethanolic extracts of fruit kernel of *Z. armatum* whereas, 37.27 ± 4.346 and 67.35 ± 4.47 per cent inhibition activity was reported in aqueous and ethanolic extracts of *P. angelicoides*, respectively. Highest FRAP activity of 0.362 ± 0.01 (mmol Fe2+ / gm of extract), followed by 0.116 ± 0.005 (mmol Fe^{2+}/gm of extract) was reported in aqueous and ethanolic extracts of *Z. armatum*.

Table 3: Per cent AICPP and FRAP activities of different extracts (Aq-aqueous; Eth-ethanolic)

Extracts	AICPP (% inhibition)	FRAP activity(mmol Fe^{2+}/gm extract)
Timur kernel (Aq)	66.97 ± 5.76[a]	0.362 ± 0.01[a]
Timur kernel (Eth)	45.71 ± 10.53[a]	0.116 ± 0.005[b]
Timur seed (Aq)	-	0.043 ± 0.005[c]
Timur seed (Eth)	-	-
Gandrani (Aq)	37.27 ± 4.34[a]	-
Gandrani (Eth)	67.35 ± 4.47[a]	-

Data are expressed as mean ± SE. Different superscripts in the same column vary significantly (p<0.05).

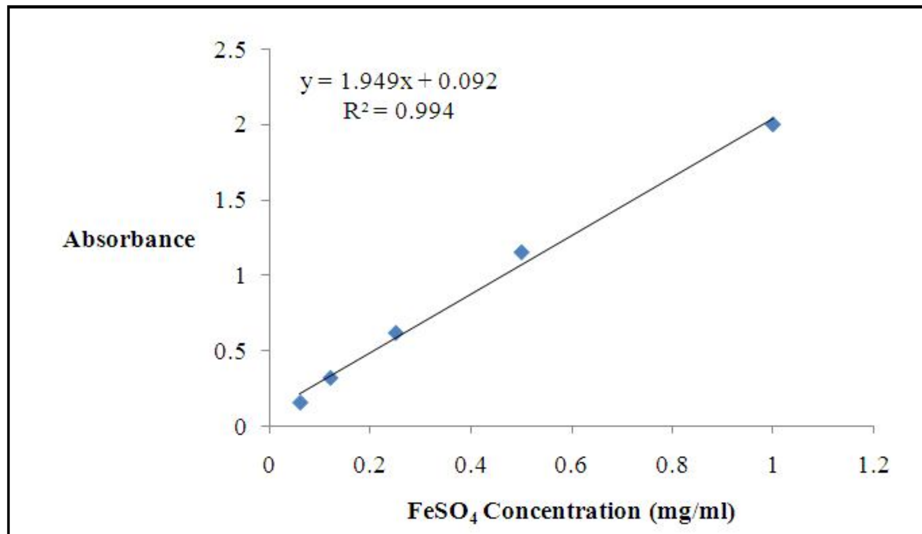

Figure 5: Linear regression curve (593 nm) for FRAP assay.

3.4 *In vitro* cytotoxicity assay

The TD_{50} concentrations of extracts are shown in Table 4. Figure 6 revealed the normal cells, DMSO control cells and effect of different extracts in MDBK cell line. TD_{50} concentrations were evaluated for aqueous and ethanolic extracts of *Z. armatum* (fruit) and *P. angelicoides* (root) in MDBK cell lines. TD_{50} concentration for both aqueous and ethanolic extracts of *Z. armatum* and aqueous extract of *P. angelicoides* was 33 µg/ml while, *P. angelicoides* (ethanolic) extract showed >1000 µg/ml TD_{50} concentration.

Table 4: TD_{50} concentrations (µg/ml) of the different extracts (Aq-aqueous; Eth-ethanolic)

S.No.	Extracts	TD_{50} concentration (µg/ml)	Non-toxic concentration (µg/ml)
1	*Z. armatum* (Aq)	33 µg/ml	10 µg/ml
2	*Z. armatum* (Eth)	33 µg/ml	10 µg/ml
3	*P. angelicoides* (Aq)	33 µg/ml	10 µg/ml
4	*P. angelicoides* (Eth)	>1000 µg/ml	1000 µg/ml

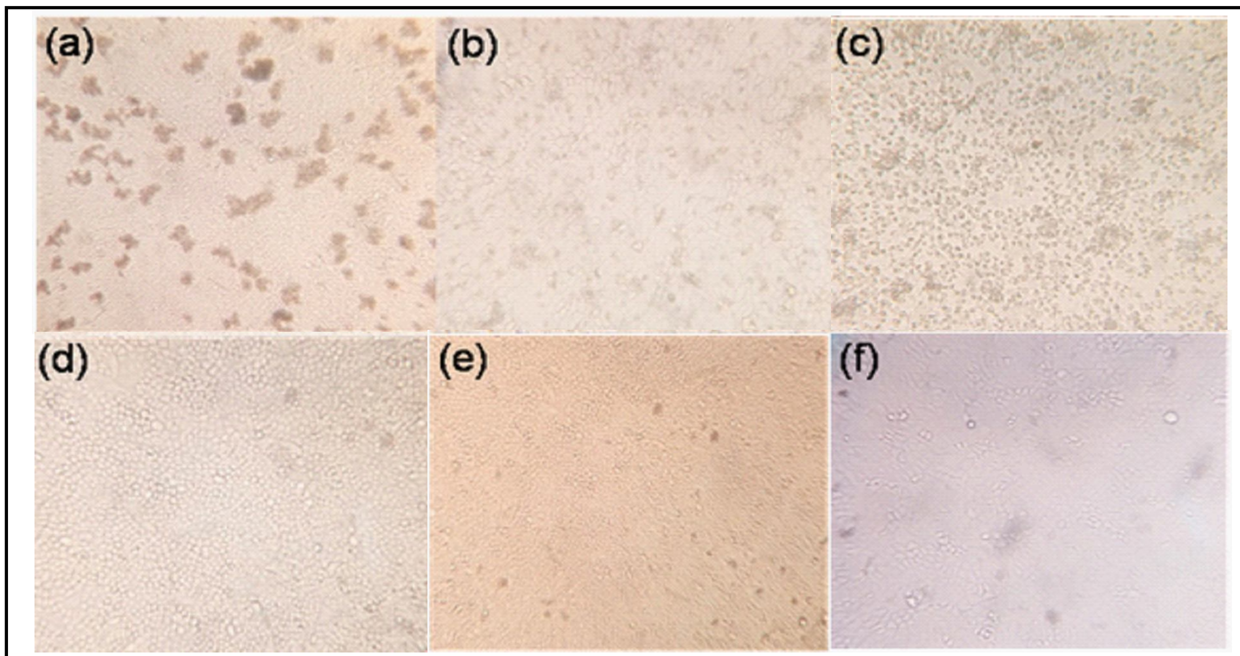

Figure 6: Effect of different treatments on MDBK cell line. (a) *Z. armatum* (Aq), (b) *Z. armatum* (Eth), (c) *P. angeliciodes* (Aq), (d) *P. angeliciodes* (Eth), (e) No treatment control, and (f) DMSO control (Aq-aqueous; Eth-ethanolic).

4. Discussion

In the present study, we have screened the presence of phytochemicals, qualitatively and quantitatively in both aqueous and ethanolic extracts of Timur (fruit kernel and seed) and Gandrani (roots). The per cent yield results of aqueous and ethanolic extracts of *Z. armatum* (fruit) and *P. angelicoides* (root) were 22.30; 30.0; 21.42 and 28.30 per cent, respectively, whereas seeds of *Z. armatum* (aqueous and ethanolic) showed 10.71 and 17.85 per cent yield only. The ethanolic fractions got a better yield than the water solvent. The low yield of seeds of *Z. armatum* may be due to the greater presence of volatile oils in it. A significant difference was observed in various parameters of different extracts of pomegranate flavedo powder using various solvents (Hamid *et al.,* 2020). Aqueous extract of *Z. armatum* (fruit kernel) revealed a strong positive reaction for flavonoids, tannins, saponins and phytosterols. An ethanolic extract of the same revealed a strong reaction for phytosterols presence only. Phytochemicals are secondary plant molecules without any nutritional benefits and are produced due to either biotic or a biotic stress factors. An earlier study has shown the presence of alkaloids, flavonoids, phenols, terpenes and coumarins in *Zanthoxylum* species (Awouafack *et al.*, 2009). Several phytocompounds comprising, terpenoids, flavonoids, alkaloids, coumarins, phenolic acids, lignins and glycosides have been reported to be present in various parts of the Timur plant. Singh and Singh (2011), reported the presence of alkaloids, phenolic compounds, saponins, flavonoids, steroids, carbohydrates, terpenes, proteins and essential oils in *Z. armatum*. Other workers also reported important phytocompounds such as flavonoids, alkaloids, phenols, coumarin, lignin, fatty acid glycosides, benzenoids and amino acids in *Z. armatum* (Ahmad *et al*., 1993, Gilani *et al*., 2010). The seeds of *Z. armatum* reported to present phytochemicals like flavonoids, tambulin and tambulol. The geneus *Zanthoxylum* is rich in alkaloids which are known to have hepatoprotective, anthelminthic, antioxidant, larvicidal, antispasmodic, antiviral, antinociceptive, antibiotic, cytotoxic, anticancer and antifungal activities (Negi *et al*., 2011). Our study revealed the phytochemicals present in the aqueous and ethanolic extracts of fruit kernel. Very few or fewer reports are available regarding the phytochemistry of fruit kernels, till today. We have reported a weak positive reaction for the flavonoids, phenols and alkaloids in the ethanolic fraction of *Z. armatum*. Flavonoids and phenolic compounds are known to have better antioxidant activity. The presence of polyphenolic compounds like tannins and flavonoids in the aqueous extract of fruit kernel make it a stronger antioxidant candidate for further exploration in various disease conditions. The aqueous extract can be a better option in the management of stress-related conditions either in humans or in animals. Timur has been used traditionally for the management of diseases like abdominal pain, fever, headache and inflammation (Mushtaq *et al*., 2019; Nooreen *et al*., 2019). Fruits, stems, leaves and bark of Timur have been documented in indigenous traditional medicine for the treatment of bloat, fever and anorexia. It is also effective to relieve colic, tooth pain and inflammatory conditions. The fruit, seeds and bark of the Timur are commonly used as a carminative, stomachic and antiparasitic drug in the traditional system of medicine. The fever and dyspepsia conditions are managed through a tonic of fruit and seeds (Thokchom and Okram, 2011). An extract of *Zanthoxylum* fruits is reported to be effective against roundworm infestation. Timur fruits have deodorant, germ killer and antiseptic properties, because of which it has been used in oral hygienic solutions and lotion for scabies (Ahmad *et al*., 1993). In the global market, the demand for *Z. armatum* is increasing due to its pharmacological relevance and its traditional background (Phuyal *et al*., 2019).

Our phytochemical analysis of *P. angelicoides* (aqueous) observed a strong positive reaction only for the alkaloids only with weak reactions for phenols, saponins and phytosterols. Alkaloids have various therapeutic applications such as antibacterials, anticancer, stimulants, antimalarial agents, anesthetics, pain killers, antihypertension agents, antispasmodics, vasodilators, antiasthma and cardiac arrhythmia. The therapeutic value and toxicity nature of the alkaloids can be an important area of research in phytomedicine (Kuete, 2014). Our experiment revealed a strong reaction for saponins with weak reactions for alkaloid and phenol presence in ethanolic extract of *P. angelicoides*. Various species of *Pleurospermum* genus contains phytoconstituents such as coumarins, saponins, flavonoids, glycosides, fatty acids and terpenoids (Rather *et al*., 2017). The genus *Pleurospermum* is rich in essential oils like germacrene, a-caryophyllene, eugenol, a-cadinene, (E)-a-farnesene, *etc*, obtained from different parts of the plant (Radulovic *et al*., 2010). Mathela *et al.* (2015) discovered that phytocompounds like angelicoidenols, a-asarone, nathoapiole, isocoumarins, 1-prophenyl-2,3,4-trimethoxybenzene, essential oils and many monoterpenes in *P. angelicoides*. A recent investigation by Ali *et al.* (2021), discovered two isocoumarins; namely, angelicoins A and B from the roots of *P. angelicoides*. The *Pleurospermum* genus possesses various medicinal activities like-analgesic (Yang *et al*., 2014), anti-inflammatory (Shepherd *et al*., 2018), anticancer (Kim *et al*., 2010), antihypertensive (Jung *et al*., 2005), antihyperlipidemic (Jung *et al*., 2007), antimicrobial (Wangchuk *et al*., 2013) and antioxidant (Mathela *et al*., 2015) activity. The strong presence of alkaloids in the aqueous extract of Gandrani makes it a suitable material for further exploration as antibacterial, antiviral and anticancer activity against different viruses.

Quantitative phytochemical analysis of aqueous and ethanolic fractions of *Z. armatum* revealed the highest total phenol and flavonoid content as compared to the aqueous extract of *P. angelicoides*. Per cent inhibition activity of AICPP was reported to be highest in the ethanolic extract of *P. angelicoides*, followed by aqueous and ethanolic extracts of fruit kernel of *Z. armatum*. Our results revealed a greater FRAP activity in aqueous and ethanolic extracts of *Z. armatum* among all extracts. *In vitro* estimation of the antioxidant potential of all the extracts using different tests revealed that the aqueous extract of *Z. armatum* has a better *in vitro* antioxidant potential compared to remaining extracts. Aqueous extract of *Z. armatum* can be employed for the management of various diseases with oxidative stress pathogenesis. The same extract can be further analyzed for the presence of different active phytoconstituents in it. However, previous studies reported the antioxidant activities through *in vitro* and *in vivo* assays in stems and bark (ethanolic) of *Z. armatum.* The *Z. armatum* extract exhibited significant antioxidant activities (Tiwary *et al*., 2011).

The ethyl acetate fraction of *Z. armatum* has shown the total phenolic content as 4.36 mg/g GAE (Minky *et al*., 2015). A recent study by Phuyal and co-workers (2020) revealed the highest TPC

and TFC value in wild fruits (226.3 ± 1.14 mg GAE/g TPC; 135.17 ± 2.02 mg QE/g TFC) of *Z. armatum* with the lowest value in cultivated seeds (137.72 ± 4.21 mg GAE/g; 76.58 ± 4.18 mg QE/g). The potent antioxidant activity was revealed in fruits of *Z. armatum.* The antioxidant potential of *Z. armatum* leaves (methanolic extract) and its solvent fractions including essential oil were evaluated with free radical scavenging activity and ferric reducing power activity. The antioxidant activity of the *Z. armatum* extract was correlated to the total phenolic content of the extracts (Guleria *et al*., 2013). The ethyl acetate fraction of *Z. armatum* revealed a higher metal chelating activity whereas, the essential oil extracted from it represents a greater reducing potential. These reports indicate the antioxidant potential of *Z. armatum*. Our study also revealed excellent antioxidant activity, TPC and TFC in fruit kernel extracts of *Z. armatum*. *Z. armatum* is a potent source of phenols, and flavonoids, suggesting its importance as a source of natural antioxidants in phytotherapy. This plant can be a better option for treating many viral infections in humans as well as in animals.

In the current investigation, TD50 concentration for aqueous and ethanolic extracts of *Z. armatum* and aqueous extract of *P. angelicoides* was calculated in MDBK cell lines. Our study showed that the ethanolic extract of *P. angelicoides* had a better safety profile as compared to the other two extracts. A previous study exhibited the cytotoxicity of essential oil from the leaves of *Z. armatum* using brine shrimp assay with IC50 values of 323 and 114 mM, respectively. In DPPH free radical scavenging assay, these compounds showed good scavenging activity (Vashist *et al*., 2016). Mathew *et al.* (2019) also studied the short term *in vitro* cytotoxicity of *Simarouba glauca* DC. in cancer cell lines and normal splenocytes with low cytotoxicity activity. In another study, ethyl acetate fraction revealed significant cytotoxic activity against lung and pancreatic cancer cell lines (A-549 lung and MIA-PaCa pancreatic cell line) with better antioxidant activity. Zanthonitrile compound isolated from *Z. armatum* exhibited a better cytotoxic activity in MTT dye assay. The IC_{50} value of zanthonitrile was recorded to be 57.28 ± 0.64 mg/ml. Zanthonitrile also exhibited antioxidant activity in a dose-dependent manner (Karmakar *et. al*., 2016). The above reports are suggestive of a better antioxidant potential of different parts of *Z. armatum*. Our study also showed the antioxidant potential of both aqueous and ethanolic fractions of *Z. armatum* (fruit kernel) using both qualitative and quantitative assays. Based on the results, the aqueous and ethanolic fractions of *Z. armatum* (fruit kernel) can be better utilized further for the amelioration of stress conditions of different natures and origins.

5. Conclusion

The present study revealed the phytochemical analysis and antioxidant activity of six different extracts of *Z. armatum* (fruit kernel and seed) and *P. angelicoides* (roots). The aqueous extract of *Z. armatum* (fruit kernel) revealed the presence of flavonoids, tannins, saponins and phytosterols with potent antioxidant activity followed by its ethanolic fraction. Aqueous and ethanolic fractions of *P. angelicoides* have reported for a stronger presence of alkaloids and saponins, respectively. The cytotoxicity assay revealed that the ethanolic extract of *P. angelicoides* (roots) is safe among all six extracts with 1000 µg/ml of maximum non-toxic concentration.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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