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Original article

Phytochemical evaluation and anticancer activity of rambutan (*Nephelium lappaceum)* fruit endocarp extracts against human hepatocellular carcinoma (HepG-2) cells

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ABSTRACT

The current investigation was taken to screen the phytoconstituents present in fruit endocarp various extracts of *Nephelium lappaceum* commonly called as Rambutan fruit and its anticancer property against human hepatocellular carcinoma (HepG-2) cells. Different analytical techniques including qualitative phytochemical analysis, cell viability assay (MTT), apoptotic nuclear staining (DAPI), DNA fragmentation assay, Attenuated total reflection (ATR) and Gas chromatography–mass spectrometry (GC–MS) spectral analysis were carried out. ATR and GC–MS study revealed the presence of functional groups and 9 compounds, respectively in methanol endocarp extract. The results obtained depicts that methanol endocarp extract profoundly controlled cell proliferation and caused shrinkage of HepG-2 cells from polygonal to spherical shape. DAPI staining revealed that methanol endocarp extract caused increased fragmentation of nucleus and DNA fragmentation, which can be taken as a sign of apoptosis. The anticancer potential of methanol fruit endocarp extract of *Nephelium lappaceum* than other extracts and could be used successfully in future drug delivery systems and other biomedical concerns.

1. Introduction

Cancer is one of the life threatening disease and holds a lot of health risk in developed and developing countries, which is also the second reason of death subsequent to cardiovascular illness (Izevbigie, 2003; Manimekalai et al., 2016a, 2016b; Rajesh et al. 2016a, 2016b; Sivakumar et al., 2019a, 2019b; Hemalatha et al., 2020). Among various cancer types, Human hepatocellular carcinoma (HCC) is the second major reason of death, and a well known malignancy that ranks fifth in men and ninth in women (Hemalatha et al., 2020). Doxorubicin a common drug for HCC treatment is effective only up to 15 to 20% as reported by Johnson (2002). Even though several treatment methods as surgical resection, radiotherapy, chemotherapy, immunotherapy *etc.,* are used, they have not been proven effective in majority of the patients and as a result, other therapeutic methods and agents, which can give less or no side effects are needed as reported by Daher et al. (2018).

The exploration of cancer remedies from natural products took place with the invention of podophyllotoxin in 1960s, and plants are probably the base for a wide range of drug formulations and 80% people from developed countries use conventional remedy as they have compounds derived from herbal plants (Sevil, 2011; Malik et al., 2018; AlSalhi et al., 2019). These drugs can act on tumour cells without harming healthy cells (Larkin, 1983; Saxe, 1987). Recent trends in drug development is focused on natural sources mainly medicinal plant parts including leaf, root, bark, stem, fruits and seeds are used in folklore medicine and there is a need for its validation as a drug to treat diseases.

Among plant parts, fruits are considered as an important source as people all around the world consume fruits. Fruits contain all

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essential phytocompounds such as such as carbohydrates, alkaloids, steroids, sterols, glycosides, flavonoids, triterpenoides, tannins, quinones, phenols, proteins and aminoacids that a plant possesses and consuming them will give good health benefits. Hence work on unravelling the phyto-constituents and health benefits of fruits has been carried out by several workers and our team has also explored the phytochemical profiling, anti-proliferative and apoptotic potential of indigenous as well as foreign fruits such as mangosteen, lemon, fig, graviola and durian on various types of cancers *in vitro* (Manimekalai et al., 2016a, 2016b; Saranya et al., 2017; Sivakumar et al., 2019a, 2019b; Hemalatha et al., 2020; Mohamad Sitheek et al., 2020). In continuation of that, the current study was taken to explore the anti-proliferative effect of rambutan fruit along with its phytochemical profiling and apoptotic potential.

Rambutan is a tropical fruit variety, widely cultivated in Southeast Asian countries. Several biological activities have been explored to unravel the natural benefits exhibited by rambutan fruit along with element composition (Nethaji et al., 2015), antimicrobial (Malini et al., 2013; Nethaji et al., 2015), antioxidant (Palanisamy et al., 2008; Thitilertdecha et al., 2008; Perera et al., 2012: Fidrianny et al., 2015), anti-diabetic (Rahayu et al., 2013; Soeng et al., 2015), anticancer (Khonkarn et al., 2010), anti-hyperglycemic (Palanisamy et al., 2011), anti-herpes simplex virus (Nawawi et al., 1999; Wirotesangthong and Rattanakiat, 2006) and anticancer (Ito et al., 2004; Mahmood et al., 2018; Hernández-Hernández et al., 2019) activities.

On the basis of the above results, we speculated anticancer properties of various extracts of the edible endocarp part of rambutan fruit on human hepatocellular carcinoma cells (HepG-2) as there are limited studies such as the protective effects of rambutan peel phenolics on oxidative damage in HepG-2 cells of mice has been reported earlier. Therefore, the present study was aimed to demonstrate the anticancer properties of rambutan fruit endocarp extracts against HepG-2 cells.

2. Materials and methods

2.1. Collection and identification

Rambutan fruits were purchased in Koyambedu market, Chennai, Tamil Nadu, India, and authenticated as per morphological characteristics as *Nephelium lappaceum* by Prof. P. Jayaraman of Plant Anatomy Research Centre, Chennai (PARC/201/4005).

2.2. Cancer cell line

HepG-2 cells (Human hepatocelluar carcinoma cells) were procured from National Centre for Cell Science, Pune, India.

2.3. Chemicals

Dulbecco's Modified Eagle Medium (DMEM), Trypsin- EDTA, Fetal Bovine Serum (FBS), 3-(4,5- Dimethyl thiazol-2yl)-2,5-dimethyl tetrazolium bromide (MTT), Dimethyl Sulphoxide (DMSO), Sodium bicarbonate, Propidium Iodide, Acridine Orange, Ethidium Bromide and Antibiotic solution were purchased from Sigma-Aldrich, USA. Likewise, 96 well plates, 6 well plates, tissue culture flasks (25 mm^2 and 75 mm^2), 15 ml and 50 ml centrifuge tubes were procured from Hi Media, USA. Chemicals used in the present study were extra pure and highest analytical quality (as given in the product).

2.4. Preparation of fruit extracts

2.4.1. Aqueous extraction

The outer epicarp of the fruits was separated and the endocarp (without seeds) was shade dried for 2 weeks at room temperature and crushed to fine powder form by maceration method using kitchen blender. A suspension of 5% (w/v) was made by adding 400 ml boiled double distilled water to 20 g of endocarp powder and keeping it in a shaker at 200 rpm for 4 h at 37 °C. Then, the suspension was cooled at room temperature, filtered throughout four layers of No.1 Whatman filter paper and at last conceded through 0.22 μm filter (Millipore, Billerica). The filtrate was freeze-dried and the fine particles were kept at −20 °C till further use (Rajesh et al., 2016a).

2.4.2. Chloroform, ethyl acetate, hexane and methanol extractions

For chloroform, ethyl acetate, hexane and methanol extract preparation, 20 g each of dried endocarp powder was taken and a suspension of 5% (w/v) was made separately by soaking it with chloroform, ethyl acetate, hexane and methanol. It was then kept for 4 h on a shaker, filtered and evaporated at room temperature in petri dishes. The dehydrated particles were retrieved and kept in respective tubes at −20 °C till further use (Rajesh et al. 2016a).

2.5. Qualitative phytochemical analysis

Qualitative tests for detecting bioactive phyto-constituents in all the five endocarp extracts of *Nephelium lappaceum* were done by using standard procedures of Kokate (1988).

2.5.1. Test for acids

Exactly 1.0 ml of fruit extract was treated with 1.0 ml sodium bicarbonate solution. Observation of effervescence designates the presence of acids.

2.5.2. Test for alkaloids

To 2.0 ml of fruit extract, 2.0 ml of HCl was added. Then few drops of Mayer's reagent were added. Formation of green colour or white precipitate indicates the presence of alkaloids.

2.5.3. Test for anthocyanin

To 0.2 ml of fruit extract, 1.0 ml of 2 N NaOH was added and heated for 5 min. at 100 ± 2 °C. The bluish green colour formed was indication for presence of anthocyanin.

2.5.4. Test for carbohydrates

To 2.0 ml of fruit extract, 1.0 ml of Molisch's reagent and few drops of H_2SO_4 was added. Presence of purple or reddish colour demonstrates the presence of carbohydrates.

2.5.5. Test for cardiac glycosides

To the fruit extract, few ml of 50% ethanol and 0.1 ml of solution of m-dinitrobenzene in ethanol and 2–3 drops of NaOH solution were added. Observation of violet colour indicates the presence of cardiac glycosides.

2.5.6. Test for coumarins

To 1.0 ml of fruit extract, 1.0 ml of 10% of sodium hydroxide was added. Formation of yellow colour indicates the presence of coumarins.

2.5.7. Test for flavonoids

To 2.0 ml of fruit extract, 1.0 ml of 2 N NaOH was added. The yellow colour formation indicates the presence of flavonoids.

2.5.8. Test for glycosides

To 2.0 ml of fruit extract, 3.0 ml of chloroform and few drops of 10% ammonia solution were added. Formation of pink colour indicates the presence of glycosides.

2.5.9. Test for phenols

To 1.0 ml of fruit extract, 2.0 ml of DH_2O followed by few drops of 10% $FeCl_3$ was added. Formation of blue or green colour indicates the presence of phenols.

2.5.10. Test for proteins

Exactly 0.5 ml of fruit extract was treated with same volume of 1% NaOH, and few drops of Cu_2SO_4 solution were gently added. Changing of the reaction mixture to purple colour indicates the presence of proteins.

2.5.11. Test for quinones

To 1.0 ml of fruit extract, 1.0 ml of concentrated $H_2SO_4$was added. Formation of red colour indicates the presence of quinones.

2.5.12. Test for saponins

To 2.0 ml of fruit extract, 2.0 ml of DH_2O was added and shaken well. The reaction mixture was kept for 15 min, where observation of 1 cm layer of foam indicates the presence of saponins.

2.5.13. Test for steroids

To 2.0 ml of acetic anhydride, 0.5 ml fruit extracts with 2.0 ml concentrated H_2SO_4 were added. Formation of violet to blue or green indicates the presence of steroids.

2.5.14. Test for tannins

To 1.0 ml of fruit extract, 2.0 ml of 5% $FeCl_3$ was added. Formation of dark blue or greenish black colour indicates the presence of tannins.

2.5.15. Test for terpenoids

To 0.5 ml of fruit extract, 2.0 ml of chloroform and 5.0 ml of concentrated H_2SO_4were added. The reddish brown colour formation indicates the presence of terpenoids.

2.5.16. Test for triterpenoids

To 1.5 ml of fruit extract, 1.0 ml of Libemann-Buchard Reagent (acetic anhydrate + concentrated sulphuric acid) was added. Turning of blue green colour of the reaction mixture indicates the presence of triterpenoids.

2.6. Anti-proliferation activity

The anti-proliferative effect of *N. lappaceum* against HepG-2 cells was determined by MTT followed by the method of Mosmann (1983). HepG-2 cells (5×10^3 cells/ml) were plated in 96 wells with serum free DMEM containing 10% FBS. The cells were incubated for 24 h under 5% CO_2 and 95% O_2 at 37°C. The medium containing cells were separated, washed with PBS and fresh DMEM medium was added and incubated for 1 h. Following the incubation, DMEM medium was discarded from control and experimental wells and 0, 7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1000 μg/ml of *N. Lappaceum* endocarp extracts (aqueous, chloroform, ethyl acetate, hexane and methanol) were added to the experimental wells containing the medium. All the samples were dissolved in DMSO (10 mg/ml) stock solution for culture studies according to the methodology of Rajesh et al. (2016a). Control cells were treated with 0.01% DMSO. The cells were then incubated for 24 h and after that, 100 μl of 0.5 mg/ml MTT was added and again incubated for 4 h. After that, 100 μl of 20% SDS in 50% dimethyl formamide (DMF) was added and pipetted 2 to 3 times smoothly to dissolve the crystals formed. Using a micro-plate reader the cell viability was read at 570 nm. The assay was also carried out for 48 h to assess the cell viability. The cell growth inhibition was calculated as follows:

$$\text{The per cent cell inhibition} = \frac{\text{OD of Experimental Sample}}{\text{OD of Control Sample}} \times 100$$

Based on the above calculation, the IC_{50} concentration of each *N. lappaceum* endocarp extract against HepG-2 was determined for 24 h and 48 h. In 24 h, IC_{50} values could not be determined and hence 48 h IC_{50} values were calculated. The least 48 h IC_{50} value against HepG-2 cells was observed in methanol fruit extract; the concentration being 63.976 μg/ml and hence 63.976 μg/ml and 1000 μg/ml concentration of methanol extract (maximum concentration) were selected for further studies.

2.7. Cytomorphological studies

The morphological changes of control and 48 h IC_{50} concentration and maximum concentration of *N. lappaceum* aqueous, chloroform, ethyl acetate, hexane and methanol endocarp extract treated HepG-2 cells were assessed. HepG-2 cells were placed in 100 mm plates and incubated for 24 h. The medium was replaced by fresh media and 48 h IC_{50} concentration of aqueous, chloroform, ethyl acetate, hexane and methanol extracts and incubated for 48 h. At the end of 48 h, the treated HepG-2 cells were visualized under inverted light microscope at 10X magnification.

2.8. Nuclear staining and apoptotic morphology

Nuclear staining using DAPI stain was done by the method of Jang et al. (2002). HepG-2 cells were plated at 1×10^6 cells/well into a six well plate. At greater than 90% confluence, the cells were treated with 63.976 μg/ml and 1000 μg/ml of methanol extract of *N. lappaceum* endocarp extract along with control cells for 48 h. The cells were washed with PBS, fixed with methanol:acetic acid (3:1, v/v) for 10 min., stained with 5 μl of DAPI stain for 20 min. and the nuclear morphology was examined under fluorescent microscope (LSM 510 Meta).

2.9. DNA fragmentation assay

N. lappaceum endocarp methanol extract (IC_{50} and maximum concentration) treated HepG-2 cells were analyzed for DNA fragmentation using the method of Wyllie (1980). Methanol extract treated (63.976 μg/ml and 1000 μg/ml) HepG-2 cells were plated in six well and kept in CO_2 incubator to achieve confluence. The cells were harvested using TPVG and 1.5 ml of cell suspension was dispensed in eppendorff tube. Cells were centrifuged at 200 × g at 4°C for 10 min., and 0.5 ml of TTE was added and centrifuged vigorously till the chromatin fragmented from the nuclei, after cell lysis due to Triton X-100 present in TTE and nuclear structure disrupted due to chelation of Mg^{++} by EDTA present in TTE. The fragmented DNA was separated from the intact chromatin by centrifuging at 20,000 × g for 10 min at 4°C.The supernatant was carefully aspirated and 500 μl of TTE and 500 μl of ice-cold sodium chloride was added and centrifuged vigorously to remove histones from DNA. Following this, 700 μl of ice-cold isopropanol was added and centrifuged and the contents were precipitated by allowing them to stand overnight at −20°C. Then, the DNA was recovered by pelleting for 10 min. at 20,000 × g at 4°C, and the pellets were rinsed in 500–700 μl of ice-cold 70% ethanol and centrifuged at 20,000 × g for 10 min. at 4°C.The DNA was then dissolved in 20–50 μl of TE and kept at 4°C. Following this, the DNA sample was mixed with loading buffer (10x) and made up to a final

concentration of 1x. The loading buffer when added to samples, allow the samples to load in well and easily monitor the run of samples. Electrophoresis was run in standard TE buffer at required voltage level. The migration of samples was monitored due to the movement of the loading dye (bromophenol blue) and when the dye reached on 3 cm before the end of the gel, the electrophoresis was terminated. The DNA was visualized by viewing the gel by UV Trans-illuminator.

2.10. ATR spectral analysis

Dried form of methanol endocarp extract of *N. lappaceum* was used in ATR spectral study. Accurately, 10.0 mg of methanol extract was encapsulated in 100 mg of KBr, to prepare translucent sample discs. The sampleswere loaded in ATR spectroscope (Brucker), with a scan range of 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1}. The results were given as graphs, which was analyzed and the functional groups were identified by the peaks and reference tables.

2.11. GC–MS spectral analysis

As the best anticancer property was observed in methanol endocarp extract of *N. lappaceum*, the phytoconstituents of methanol endocarp extract with its structure was assessed by GC–MS chromatogram. The filtrate was analyzed for secondary metabolites by using GC MATE II GC–MS (Agilent). For this, 1.0 µl of the compound was injected through HP-5 capillary column, maintained at 220°C and helium was used as carrier gas at a flow rate of 1.0 ml/min. After analysis, the compounds were identified with the help of structural library.

2.12. Statistical analysis

The mean and its standard error of five individual observations obtained from MTT assay were calculated along with the per cent change between untreated and treated data. 'Two Way ANOVA' using SPSS software was used to determine the significance between the treatments and concentrations at $p < 0.05$ level; the data being presented the form of tables and figures in results section (Rajesh et al., 2016a).

3. Results

The dry endocarp extract of *N. lappaceum* is highly soluble in distilled water, chloroform, ethyl acetate, hexane and methanol and all the five endocarp extracts of *N. lappaceum* appeared as dark brown colored paste. The colour of extracts was dark brown when viewed by human eye and the consistency was paste when touched by hands. The yield of *N. lappaceum* endocarp extracts was maximum in methanol (5 g), followed by aqueous (2.5 g), ethyl acetate (2 g) hexane (1 g) and chloroform (1 g); the data being presented in Table 1.

The qualitative phytochemical analysis showed the presence of alkaloids, carbohydrate, cardiac glycosides, flavonoids, protein, steroids, and triterpenoids in aqueous extract. Likewise, alkaloids, carbohydrates, coumarins, saponins and tannins were present in chloroform extract. On the other hand, carbohydrate, cardiac glycosides, steroids and terpenoids were present in ethyl acetate extract. Alkaloids, carbohydrate, cardiac glycosides, coumarins, saponins, steroids, tannins and terpenoids were present in hexane extract, and alkaloids, carbohydrate, cardiac glycosides, coumarins, flavonoids, phenols, protein, saponins, tannins, terpenoids and triterpenoids were present in methanol extract (Table 2).

The functional group of the active components was identified using ATR based on the peak value in the area of infrared radiation band. The outcome of ATR peak values and functional groups revealed the presence of alkanes, alkenes, alkynes, aldehydes, alkyl halides, aromatics, carboxylic acids, esters, nitro compound, phenols and phosphine in the methanol endocarp extract (Table 3 and Fig. 1).

The methanol endocarp extract of *N. lappaceum* was anlayzed by GC–MS, which revealed 9 compounds with their respective RT and peak name and with NIST/NBS spectral database (Table 4 and Fig. 2). The compounds obtained were 4H-pyran-4-one,2,3-

Table 1
Yield of solvent extracts of *N. lappaceum*.

Solvents	Weight of dried powder (g)	Yield dried extracts (g)	Colour	Consistency
Aqueous	100	2.5	Dark brown	Paste
Chloroform	100	1	Dark brown	Paste
Ethyl acetate	100	2.0	Dark brown	Paste
Hexane	100	1	Dark brown	Paste
Methanol	100	5.0	Dark brown	Paste

Table 2
Qualitative phytochemical analysis of various endocarp extracts of *N. lappaceum*.

Secondary metabolites	Aqueous	Chloroform	Ethyl acetate	Hexane	Methanol
Acids	–	–	–	–	–
Alkaloids	+	+	–	+	+
Anthocyanin	–	–	–	–	–
Carbohydrate	+	+	+	+	+
Cardiac glycosides	+	–	+	+	+
Coumarins	–	+	–	+	+
Flavonoids	+	–	–	–	+
Glycosides	–	–	–	–	–
Phenols	–	–	–	–	+
Protein	+	–	–	–	+
Quinones	–	–	–	–	–
Saponins	–	+	–	+	+
Starch	–	–	–	–	–
Steroids	+	–	+	+	–
Tannins	–	+	–	+	+
Terpenoids	–	–	+	+	+
Triterpenoids	+	–	–	–	+

+: Positive; - : Negative.

Table 3
AT-IR spectral peak values and functional groups (4000 to 600 cm^{-1}) obtained for methanol endocarp extract of *N. lappaceum*.

Peak Value (cm^{-1})	Functional groups
767.60	Alkyl halides (C-Cl stretch)
816.14	
861.68	Aromatics (C-H out of plane)
925.19	Carboxylic acid (RCOOH O-H bend)
1014.78	Ethers (C-O stretch)
1347.34	Nitro compound (N-O aromatic)
1411.20	Aromatics (Ar C-C stretch)
1637.73	Alkenes (C = C stretch)
1729.54	Esters (C = O stretch)
2137.36	Alkynes (C#C stretch)
2930.72	Alkanes (CH stretch)
3293.81	Phenols (ArO-H H-bonded)

Note: The functional groups were identified by the peaks and ATR reference tables.

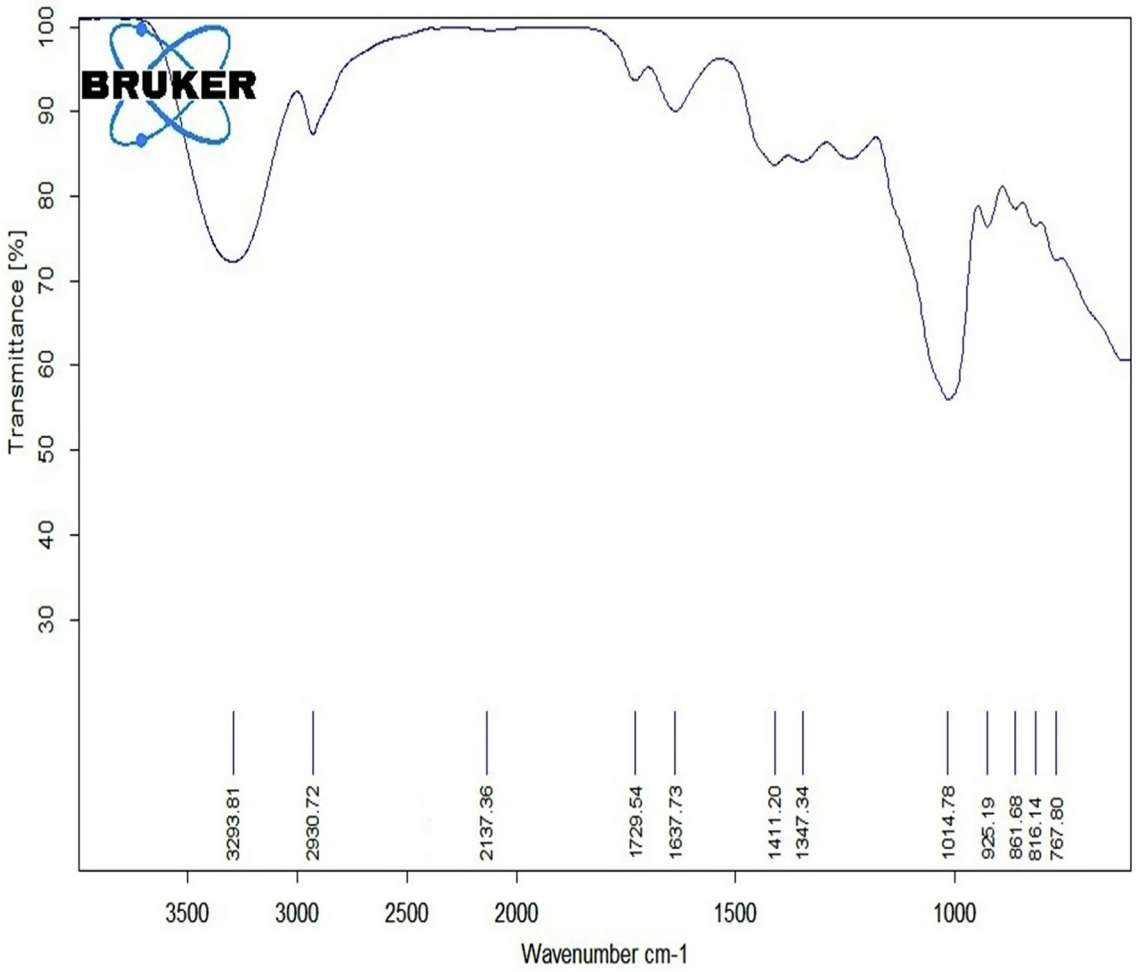

Fig. 1. ATR spectra of various functional groups (4000 to 600 cm^{-1}) obtained for methanol endocarp extract of *N. lappaceum.*

Table 4
GC–MS Spectral analysis of *N. lappaceum* methanol endocarp extract.

RT	Name of the compound
10.99	1-(nitromethyl)-cyclohexanol
13.53	4H-pyran-4-one,2,3-dihydro-3, 5-dihydroxy-6-methyl
15.57	2-furancarboxaldehyde, 5-(hydroxyllate)-
17.17	T-butyl cyclopentaneperoxycarrboxylate
19.91	4-pentylcyclohexanone
20.29	Pyrimidine-2,4,6(1H,3H,5H)-troine, 1-octadecyl
21.30	2-carbamyl-9-(Beta-D-ribofuranosyl) Hypoxanthine
26.90	Pseduosarsasapogenin-5, 20-dien methyl ether
28.45	11-eicosenoic acid, trimethylsilyl ester

Note: The compounds were identified with the help of structural library.

dihydro-3, 5-dihydroxy-6-methyl, 2-furancarboxaldehyde, 5-(hydroxyllate)-, T-butyl cyclopentaneperoxycarrboxylate, 4-pentylcyclohexanone, Pyrimidine-2,4,6(1H,3H,5H)-troine, 1-octadecyl, 2-carbamyl-9-(Beta-D-ribofuranosyl) Hypoxanthine, Pseduosarsasapogenin-5, 20-dien methyl ether and 11-eicosenoic acid, trimethylsilyl ester.

Anti-proliferation of HepG-2 cells was assessed by MTT for 24 h and 48 h in all the five endocarp extracts of *N. lappaceum.* In 24 h, anti-proliferation was not observed. In 48 h treatment, anti-proliferation was seen in the HepG-2 cells while treated with various concentrations (0 (control), 7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1000 μg/ml) of all the five endocarp extracts. The control cells were 100% viable, while treated cells recorded decreased cell viability; the decrease being indirectly proportional to concentration. Statistical testing of the data by two-way ANOVA indicated that all the values were significant at $p < 0.05$ (Table 5 and Fig. 3).

The IC_{50} value of cell viability of HepG-2 cells could be obtained for all the extracts only in 48 h treatment. The IC_{50} value was 98.574 μg/ml in aqueous extract, 661.778 μg/ml in chloroform extract, 183.111 μg/ml in ethyl acetate extract, 180.041 μg/ml in hexane extract and 63.976 μg/ml in methanol extract at 48 h incubation (Table 6). Among all the extracts, the methanol endocarp extract showed higher activity, which obviously shows that methanol endocarp extract has profound effect in controlling HepG-2 cell proliferation. The data *in toto* depicts that methanol endocarp extract of *N. lappaceum* controls cell proliferation of HepG-2 cells.

The morphological observation of HepG-2 cells is presented in Fig. 4 (Plate 1). The control HepG-2 cells showed irregular confluent aggregate as rounded and polygonal cell shape. On the other hand, treatment of the cells with *N. lappaceum* endocarp extracts for 48 h resulted in shrinkage of polygonal cells and changed to spherical shape. The cell shrinkage was progressive; the changes being dose and time dependent. The rate of the shrinkage was high in methanol endocarp extract than that of its counterparts. This might be due to the presence of phytochemicals in methanol endocarp extract.

To find out whether the cytotoxic effects induced by methanol endocarp extract of *N. lappaceum* involves apoptotic changes, the nuclear condensation was observed by the DAPI staining. A very

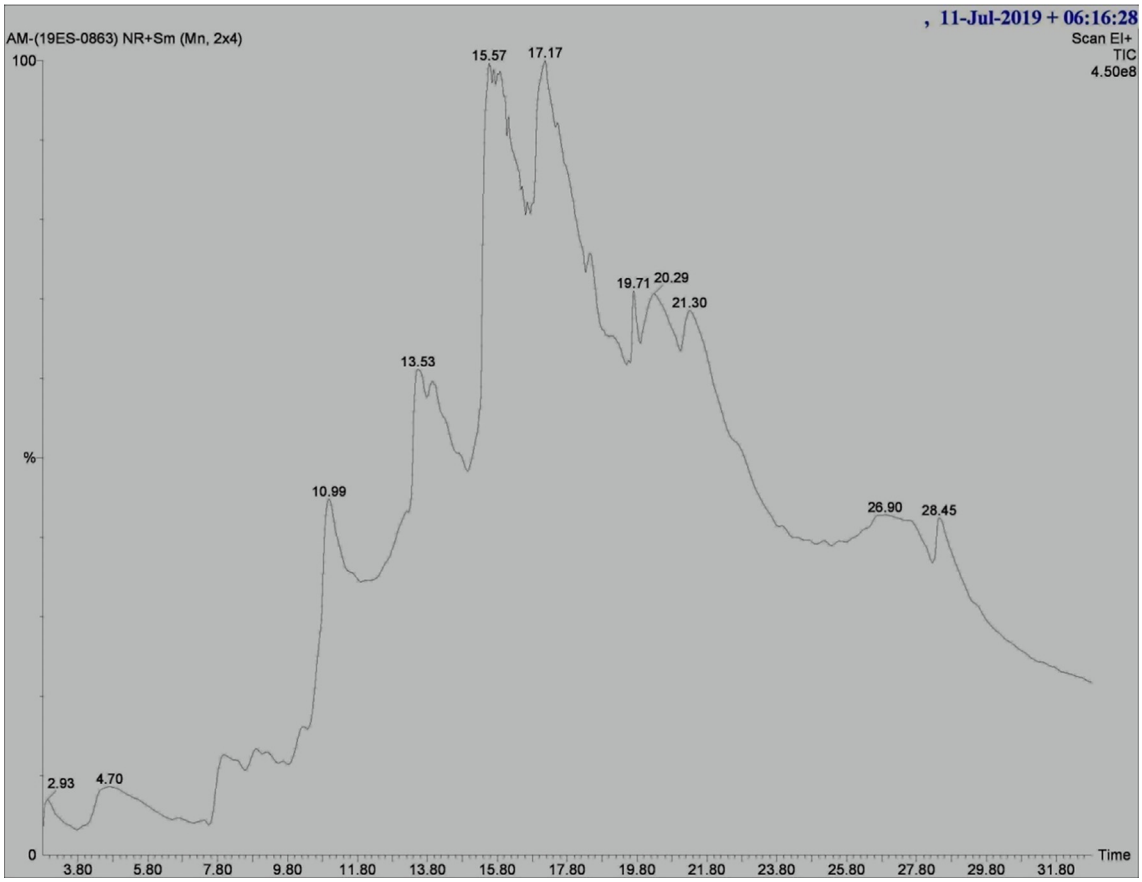

Fig. 2. GC–MS chromatogram of *N. lappaceum* methanol endocarp extract.

Table 5
Per cent cell viability of HepG-2 cells when treated with various endocarp extracts of *N. lappaceum* for 48 h.

Concentration(μg/ml)	Samples				
	Aqueous	Chloroform	Ethyl Acetate	Hexane	Methanol
Control (0)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
7.8	88.89 ± 1.51*(−11.111)	94.29 ± 0.41* (−5.708)	89.14 ± 0.84* (−10.856)	91.85 ± 0.06* (−8.154)	77.46 ± 0.77* (−22.545)
15.6	76.37 ± 0.82* (−23.630)	86.16 ± 0.69* (−13.844)	77.46 ± 0.86* (−22.541)	82.38 ± 1.51* (−17.622)	70.39 ± 0.95* (−29.606)
31.2	67.17 ± 1.56* (−32.830)	76.39 ± 1.02* (−23.612)	69.85 ± 0.89* (−30.153)	77.75 ± 1.12* (−22.249)	63.06 ± 1.02* (−36.941)
62.5	55.16 ± 0.72* (−44.839)	69.05 ± 0.93* (−30.955)	59.52 ± 0.89* (−40.475)	72.05 ± 1.31* (−27.953)	50.27 ± 0.72* (−49.731)
125	46.22 ± 1.08* (−53.782)	61.42 ± 0.47* (−38.584)	53.29 ± 1.24* (−46.70)	55.99 ± 0.86* (−44.001)	38.88 ± 0.98* (−61.125)
250	35.61 ± 0.64* (−64.391)	52.46 ± 0.98* (−47.540)	46.20 ± 0.81* (−53.795)	42.37 ± 0.64* (−57.625)	26.62 ± 0.64* (−73.378)
500	21.21 ± 1.09* (−78.786)	44.86 ± 0.93* (−55.144)	37.78 ± 0.57* (−62.222)	33.18 ± 1.35* (−66.815)	13.85 ± 0.72* (−86.147)
1000	11.71 ± 0.91* (−88.293)	39.97 ± 0.99* (−60.028)	29.89 ± 0.83* (−70.112)	27.72 ± 0.69* (−72.276)	6.80 ± 0.56* (−93.199)

Values are mean ± S.E. of five observations; Values in parentheses are per cent change over control; -Donates % decrease over control; * Denotes that values are significant at $P < 0.05$.

negligible amount of positive cells were present in control HepG-2 cells, while treatment of HepG-2 cells for 48 h with 63.976 μg/ml (48 h IC_{50} concentration) and 1000 μg/ml (maximum concentration) of methanol endocarp extract increased positive cell counts. The nucleus was intact in control HepG-2 cells, while methanol endocarp extract of *N. lappaceum* at IC_{50} 48 h and maximum concentration caused increased fragmentation of nucleus, which can be taken as a sign of apoptosis and was well indicated by DAPI staining shows in Fig. 5 (Plate 2).

Agarose gel electrophoretic pattern showing DNA fragmentation of HepG-2 cells is depicted in Fig. 6 (Plate 3). Control HepG-2 cells showed the presence of intact genomic DNA in the wells or just below the wells with no characteristic laddering pattern. On contrary, methanol endocarp extract treated cells showed characteristic laddering pattern in 100 to 200 bp at 48 h IC_{50} concentration and 200 to 300 bp at maximum concentration, which shows that the methanol endocarp extract of *N. lappaceum* effectively induces DNA fragmentation in treated cells than control.

4. Discussion

The phytochemical analysis and structural determination of phytoconstituents present in *N. lappaceum* has been reported by Thitilertdecha et al. (2010), Nethaji et al. (2015) and Sukumandari et al. (2017). Nethaji et al. (2015) reported that the methanolic extracts of *N. lappaceum* fruit epicarp showed the presence of carbohydrates, proteins and aminoacids, steroids, alkaloids, flavonoids, tannins, triterpenoid and glycosides. The most impor-

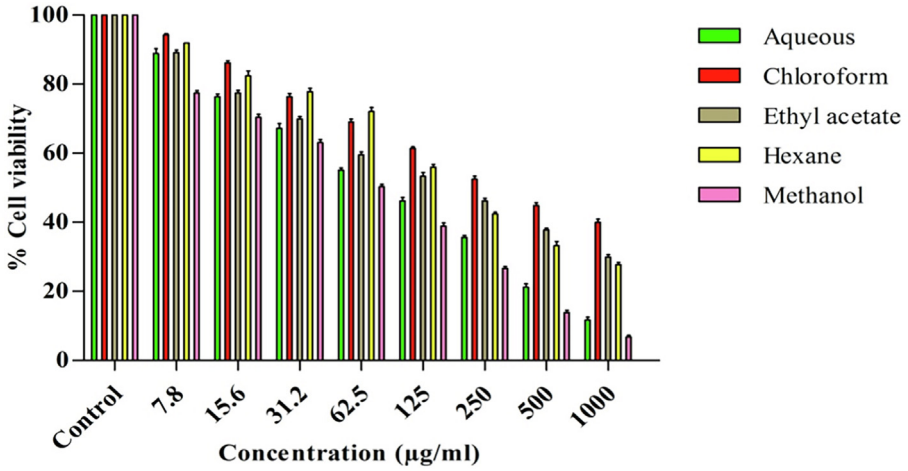

Fig. 3. Cell viability by of HepG-2 when treated with *N. lappaceum* endocarp extracts for 48 h.

Table 6
IC_{50} values of HepG-2 cells when treated with various endocarp extracts of *N. lappaceum* for 48 h.

Sample (Extract)	IC_{50} Value (µg/ml)
Aqueous	98.574
Chloroform	661.778
Ethyl acetate	183.111
Hexane	180.041
Methanol	63.976

tant component reported is "geraniin" as of the rind of *N. lappaceum,* which exhibited significant biomedical property by mitigating obesity-induced metabolic dysfunction. Geraniin has proved effective against dengue virus type-(DENV-2) as reported by Thitilertdecha et al. (2010) and Sukumandari et al. (2017).

The anticancer property of methanol extract of red and yellow varieties of *N. lappaceum* against breast cancer cells (MDA-MB-231), cervical cancer cells (HeLa) and osteosarcoma cancer cells (MG-63) were reported by Khaizil et al. (2013). The results indicated that yellow variety gave an IC_{50} value 5.42 ± 1.67 µg/ml and 6.97 ± 1.0 µg/ml for MDA-MB–231 and MG-63 with, respectively. On the other hand, extracts of all varieties did not illustrate any anticancer effects towards HeLa cells. Likewise, Chunglok et al. (2014) reported anticancer activities of the methanol extracts from seeds and pericarps of three selected tropical fruits including *N. lappaceum*, *Litchi chinensis* and *Tamarindus indica* on human mouth carcinoma (CLS-354) cells. Results of the study indicated high activity of tamarind followed by litchi and rambutan against CLS-354 cells, and these finding supports our results too, indicating that methanol endocarp extract of rambutan has anti-proliferative effect on HepG-2 cells.

Apoptosis is a very important process including normal cell turnover, suitable immune system growth and function, hormone-dependent atrophy, embryonic development and chemical-induced cell death. In addition, irregular apoptosis is connected with several human physiological conditions, such as neurodegenerative diseases, ischemic damage, autoimmune disorders and various types of cancers (Cho et al., 2015). Induction of apoptosis is the key to success of plant products as anticancer agents (Hsiao and Liu, 2010; Devanesan et al. 2020; Hemalatha et al., 2020). When the cytotoxic effects induced by methanol endocarp extract of *N. lappaceum* was checked for its involvement in apoptotic changes by analyzing nuclear morphological changes by the DAPI staining, the nuclear condensation was observed. To support our results, the Abdolmohammadi et al. (2008) also reported that T47D cells treated with the *Astrodaucus persicus* extracts showed reduced cell proliferation when analysed by DAPI staining.

The present study on DNA fragmentation showed that methanol endocarp extract of *N. lappaceum* effectively induced DNA fragmentation in treated cells than control. This might be due to the fact that DNA is cleaved in more consequent by the standard agarose gel electrophoresis. The arrangement of the DNA ladder in gel electrophoresis indicated that induction of apoptosis in HepG-2 cells has been initiated by the phytochemicals present in rambutan methanol extract.

In our investigation, cytotoxic activity of rambutan methanol endocarp extract to HepG-2 cells could have caused apoptosis induction in the cells as depicted by DNA fragmentation studies. The capacity of the extract to induce apoptosis could be due to the presence of secondary metabolites that are present in the endocarp methanol extract of rambutan. Cell viability analysis and MTT tests depicted that methanol endocarp extract of rambutan might have induced apoptosis in HepG-2 cells leading to the decrease in viability. Similar study have been done by Saranya et al. (2017) in MCF-7 cells when treated with *Citrus limon,* Flora Priyadarshini et al. (2020) in HepG-2 cells when treated with propolis and Hemalatha et al. (2020) in HepG-2 cells when treated with *Annona muricata. In toto*, the data tells that methanol endocarp extract induced death of HepG-2 cells through apoptotic pathway, which finds support from the above authors.

The phenolic and flavonoid compounds induced apoptosis *via.,* cell cycle arrest progression, increasing pro-protein (Bax and Bad) levels and decreasing anti-apoptotic protein levels (Bcl-2 and Bcl-xL) in the HepG2 cells as demonstrated by Granado-Serrano et al. (2006), Ho et al. (2009), Granado-Serrano et al. (2010), Naowaratwattana et al. (2010), Haza and Morales (2011) and Janicke et al. (2011). Therefore, the presence of phenolic and flavonoid compounds in rambutan fruit might have attributed to the anticancer activity of the rambutan methanol endocarp extract against HepG-2 cells.

The functional group of the active components present in methanol endocarp extract was identified using ATR based on the peak value in the area of infrared radiation band. The outcome of ATR peak values and functional groups revealed the presence of alkanes, alkenes, alkynes, aldehydes, alkyl halides, aromatics, car-

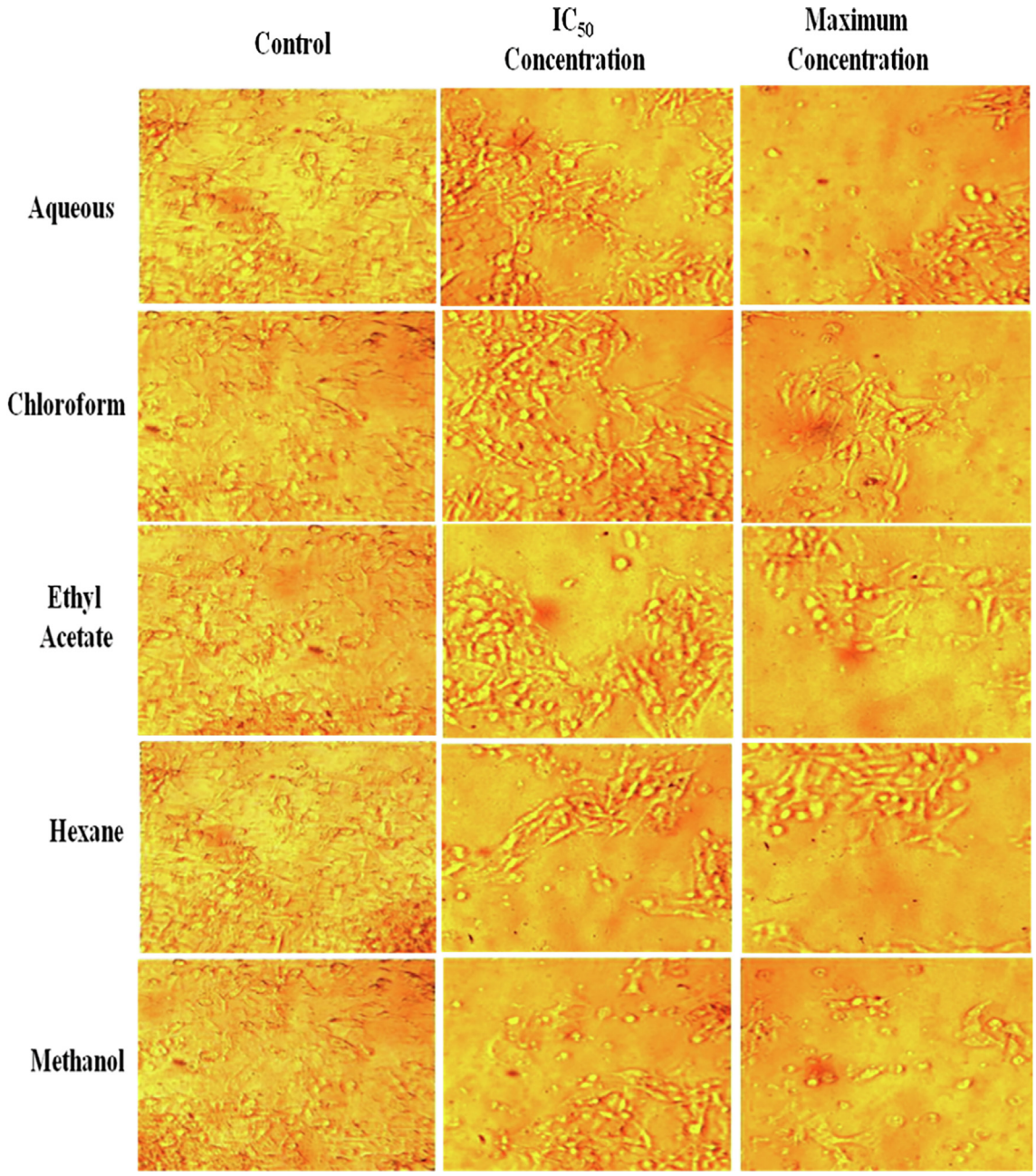

Fig. 4. (Plate 1) Cell morphology of HepG-2 cells when treated with IC_{50} concentration and maximum concentration of *N. lappaceum* endocarp extracts for 48 h.

boxylic acids, esters, nitro compound, phenols and phosphine in the methanol endocarp extract. Similar analysis of rambutan husk by Méndez-Flores et al. (2018) also indicated the presence of the main functional groups of polyphenolic compounds such as hydroxyl groups, carboxylic acids, aromatic rings and carbonyl group in rambutan husk, which is similar to our results.

The methanol endocarp extract of *N. lappaceum* was anlayzed by GC–MS, which revealed 9 compounds with their respective RT and peak name and with NIST/NBS spectral database. A similar study of Ong et al. (1998) supported our arguments on rambutan fruit were identified more than 100 volatiles compounds by GC/MS. Mostly were odour compounds out of that 20 compounds were stronger odorants such as beta-damascenone, (E)-4,5-epoxy-(E)-2-decenal, vanillin, (E)-2-nonenal, phenylacetic acid, cinnamic acid, unknown 1 (sweaty), ethyl 2-methylbutyrate, and delta-decalactone. Depending on the odour activity values, beta-damascenone, ethyl 2-methylbutyrate, 2,6-nonadienal, (E)-2-nonenal, and nonanal were determined to be the major contributors to the aroma of the fruit.

5. Conclusion

In this study, we have found out that rambutan endocarp extracts, predominantly the methanol extract possess strong anticancer activity against human hepatocellular carcinoma (HepG-2) cells. The results pragmatically shows that major polyphenolic compounds like phenols and flavonoids present in methanol endocarp extract are most likely responsible for the anticancer and apoptotic activity. In future studies, we have planned to isolate the active principle compounds present in methanol endocarp

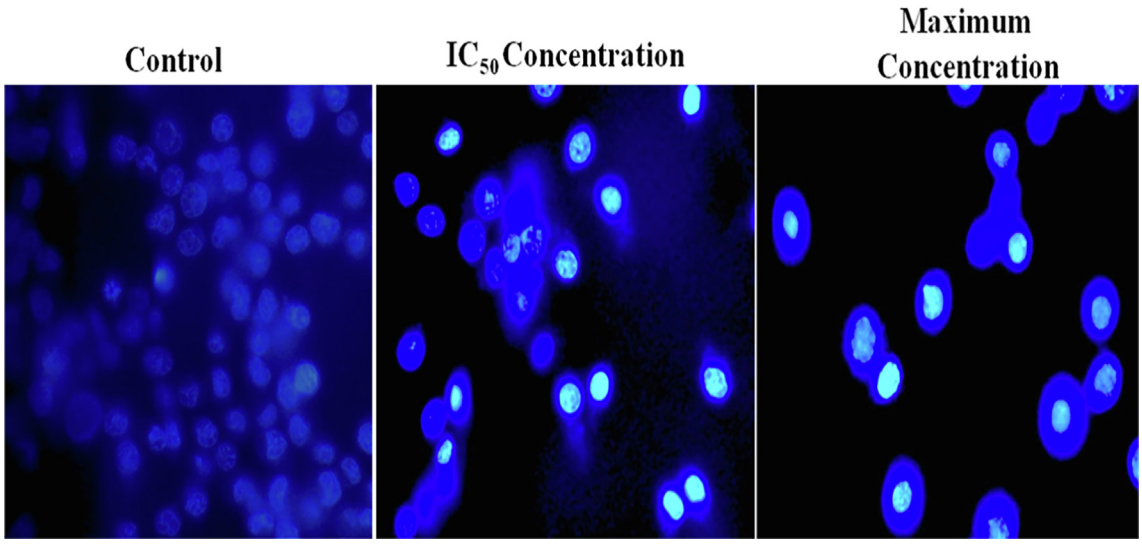

Fig. 5. . (Plate 2): Fluorescence microscopic images showing nuclear morphology of control IC_{50} concentration and maximum concentration of *N. lappaceum* methanol endocarp extract treated HepG-2 cells for 48 h.

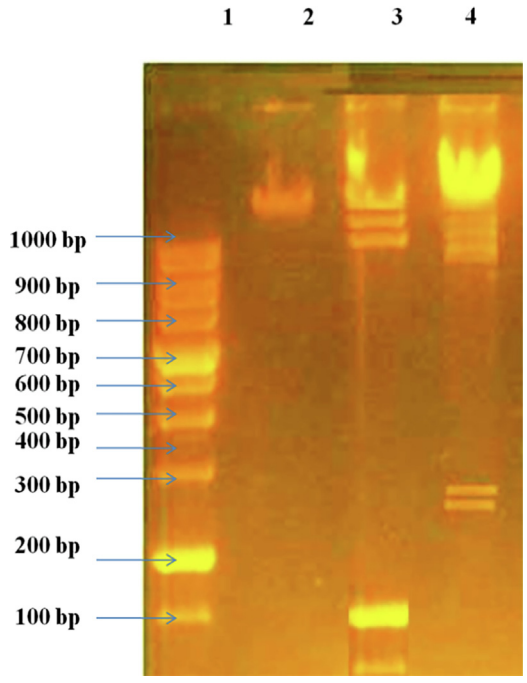

Fig. 6. . (Plate 3): Quick detection of apoptotic DNA ladder in HepG-2 cell line. Lane 1: Standard molecular marker (1000 bp). Lane 2: Control HepG-2 cells. Lane 3: IC_{50} concentration of *N. lappaceum* methanol endocarp extract treated HepG-2 cells. Lane 4: Maximum concentration of *N. lappaceum* methanol endocarp extract treated HepG-2 cells.

extracts of rambutan and assess its potential as an anticancer agent.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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