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In vitro studies on antimicrobial and antioxidant effect of methanolic extract of *Indigofera aspalathoides* (Vahl ex DC) and its cytotoxic property against human lung cancer cell line NCI H460

S. Rajaperumal, M. Nimmi and B. D. Ranjitha Kumari*

Department of Plant Science, Bharathidasan University, Tiruchirapalli, India

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ABSTRACT

The present study has been under taken with an objective to determine the antimicrobial, anti oxidant and cytotoxic activity of the methanolic root extract of Indiofera aspalathoides (Vahl ex Dc) by using different solvents(methanol, ethanol, aqueous). The selected medicinal plant was collected from the campus of Bharathidasan University, Tiruchirappalli. Antibacterial activity was carried out against gram positive and gram negative bacteria. Antioxidant property was determined quantitatively. Determination of cytotoxic activity of the methanolic root extract was carried out on human lung cancer cell line NCL H460.Among the different solvent extracts used in the study, the methanolic root extract showed highest antimicrobial and cytotoxic activity against microbes and human cancer cell line respectively. The extract screened for phytochemical analysis was found to contain bioactive compounds like steroids, saponins, flavonoids, terpenoids, phenolic compound, anthraquinones, tannins and reducing sugars. The methanolic root extract inhibited the growth of all bacterial and fungal pathogens tested. Among the bacterial species tested (Salmonella typhi, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes and Pseudomonas aeruginosa),highest zone of inhibition was found in K. pnemoniae (1.8 cm) and among the fungal pathogens tested (Aspergillus niger, A. flavus, A. fumigatus, Candida albicans), highest inhibition zone was noticed against A. fumigates (1.6 cm). The strong antioxidant activity was observed at 20µg/ml with an IC 50 value of 18.1 µg/mL higher than that of the standard (Ascorbic acid) .The cytotoxic effect of methanolic root extract on lung cancer cell line NCL H460 was studied. Thin layer chromatography analysis of the methanolic root extract showed 5 compounds. The percentage of cell viability was found to decrease with increasing concentration of the extract. This study suggests that methanolic root extract of Indigofera aspalathoides have profound antimicrobial, antioxidant and cytotoxic effect.

Key words: *Indigofera aspalathoides*, antimicrobial, antioxidant, cytotoxic activity, phytochemical analysis, TLC.

INTRODUCTION

Medicinal herbs have a long history in improving human health and curing various diseases. A wide interest has been made for researchers using herbal material in identification of the active components and verification of their efficiency. All modern clinical drugs over 50 % are of natural product origin. India has an extensive rich heritage of herbal medicine since from the time of ayurveda with medicinal properties. There are three ways in which plants have been found useful in medicine. First, they may be used directly as teas or in other extracted forms for their

natural chemical constituents. Second, they may be used as agents in the synthesis of drugs. Finally, the organic molecules found in plants may be used as models for synthetic drugs. Historically, the medicinal values of plants were tested by trial and error, as in the Doctrine of Signatures (Gayathiri and Uma, 2009).

Various medicinal properties have been attributed to natural herbs. Medicinal plant constitutes the main source of new pharmaceuticals and healthcare products. Due to enormous propensity of plants, which synthesize a variety of structurally diverse bioactive compounds; the plant kingdom is a potential source of chemical constituents with antitumor and cytotoxic activities (Kim *et al.*, 2005).

Indigofera aspalathoides Vahl ex DC, belonging to the family Fabaceae is a distributed shrub found throughout India which is commonly known as 'Shivanar Vembu' in Tamil. It is a small trailing much branched annual or biennial herb and is a reputed indigenous medicine. The aerial parts of the plant have shown to possess antihepatoxic activities. The alcoholic extract of the leaves possess antihepatoxic effect against D-galactosamine and carbon tetra chloride induced damage in liver (Sreepriya *et al.*, 2001). In the traditional medicinal system, the leaves, flowers and tender shoots are said to be cooling and demulcent; they are used in the form of decoction for leprosy and cancerous affections (Kirtikar and Basu, 1975**)**. The aerial parts of *Indigofera tinctoria* was used in treatment of anti-proliferative activity in human non small cell lungs cancer cell A-549 (Kameswaran and Ramanibai 2008).

Methanolic extract of *I. aspalathoides* shows both antibacterial and antifungal activity. Root extracts of *I. aspalathoides* has very good antimicrobial activity **(**Jeyachandran *et al*., 2011). This plant may be used for treatment of several diseases caused by pathogenic microbes. This study recommends future research regarding the pharmacological investigations (drug designs) of this plant (Natarajan *et al*., 2010). A study with stem extract indicates that it has antitumor, antiviral and antibacterial effect (Philips *et al.*, 2010).

With this background the present study was carried out with an aim of evaluating the antimicrobial, antioxidant and anticancer activity of the root extract of *Indigofera aspalathoides using* different solvents.

MATERIALS AND METHODS

Collection of medicinal plant
Fresh plants *of Indigofera aspalathoides* was collected from Bharathidasan University, Tiruchirappalli.

Preparation of leaf extract
The plant was collected, cleaned with water and dried in room temperature for one week. Then they were ground into fine powder using herbal grinding mill and stored in room temperature. The air dried and powdered root of plant material (20 g) was extracted with 200 ml of methanol using soxhlet apparatus for 24 hrs. The extract was filtered and evaporated until dryness. The extract was stored at 4°C until further use.

Preliminary Phytochemical Screening
Phytochemical screening for alkaloids, flavanoides, quinines, glycosides, terpenoids and saponins were analyzed using the powdered methanolic root extract using the method of Brindha *et al*., 1982.

Thin layer chromatography:
It is used to separate the compound present in the crude extract. The solvent used is 20 % methanol. The concentration (10 mg/ml) of the drug was spotted on the TLC plates and dried. It was then run with different solvent ratio. The spots were identified in the UV light, far light and in the iodine chamber. Then Rf value was calculated.

Antimicrobial activity of *Indigofera aspalathoides*:
Antimicrobial activity was screened by agar well diffusion method. The methanolic root extract was tested for antimicrobial activity against bacterial pathogens (*Salmonella typhi, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa*) and fungal pathogens (*Aspergillus niger, A. flavus, A. fumigatus, Candida albicans).*

Antioxidant activity of the crude extract of the root of *Indigofera aspalathoides:*
Antioxidant activity of the crude extract of the root of *Indigofera aspalathoides* was determined on the basis of its scavenging potential of the stable DPPH (1, 1-diphenyl 1 2-picryl hydrazyl) free radical.

DPPH assay was performed based on the measurement of the scavenging ability of antioxidant towards the stable DPPH radical and the scavenging activity was determined by the method of Sreejayan *et al*.,(1996). The free radical DPPH is purple in color in methanol and is reduced to corresponding hydrazine, which is yellow in color, when reacts with hydrogen donor. To 200 µl of 100 µM DPPH solution, 10 µl of various concentrations of the extract or the standard solution (Ascorbic acid) was added separately in wells of the microtitre plate. The plates were incubated at 37°C for 30 min. Absorbance was measured at 517 nm using ELISA reader.

The optical density was recorded and (%) inhibition was calculated using the formula:

$$\% \text{ Inhibition of DPPH activity} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Hydroxyl radical scavenging activity assay

This assay was carried out according to the method of Halliwell *et al* (1987). To the reaction mixture containing deoxyribose (3 mM, 0.2 ml), ferric chloride (0.1 mM, 0.2 ml), EDTA (0.1 mM, 0.2 ml), ascorbic acid (0.1 mM, 0.2 ml) and hydrogen peroxide (2 mM, 0.2 ml) in phosphate buffer (pH, 7.4, 20 mM), 0.2 ml of various concentrations of extracts or standards in DMSO were added to give a total volume of 1.2 ml. The solutions were then incubated for 30 min at 37°C. After incubation, ice- cold trichloro acetic acid (0.2 ml, 15 % w/v) and thiobarbituric acid (0.2 ml, 1 % w/v), in 0.25 N HCl were added. The reaction mixture was kept in a boiling water bath for 30 min, cooled and the absorbance was measured at 532 nm.

Cytotoxic activity of *Indigofera aspalathoides* on lung cancer cell line:

The cytotoxic effect of methanolic root extract of *Indigofera aspalathoides* against NCL H460 was assayed by MTT assay. NCI H460 cells were grown in 96 well micro titer plate (5 x 10^3cells/well) for 24 hr after seeding. The plates were incubated with methanolic extract at different concentrations for 24 and 48 hours respectively. The medium were refreshed and 20µl of MTT (5 mg/ml) was added. The plates were incubated for three hr in dark. The formazan crystals developed were solublized with100µl of DMSO and the plate was kept in dark for another 5-10 min. The color developed was measured in an ELISA reader (Bio Rad, USA) at wavelength 570 nm and with reference wavelength at 630 nm.

A graph of absorbance (Y- axis) against the concentration of the drug (X-axis) was plotted and the IC_{50} concentration was determined as the dye concentration that was required to reduce the absorbance to half that of control. The data was then converted to percentage inhibition curve, to normalize a series of curves.

Assessment of Cell Morphology

The Assessment of Cell Morphology was done using Acridine Orange & Ethidium Bromide staining method. NCI h460 cells were grown in 6 well plates (5x10^3cells/well) for 24 hr. The cells were then incubated with the IC_{50} dose of extract for 24 and 48 hrs. The medium was discarded and the cells were washed with PBS. The cells were then trypsinized and placed on a glass slide and stained with acridine orange & ethidium bromide, or Hoechst stain. The cells were then viewed in an epifluorescent microscope.

DNA fragmentation analysis

Cells were span down at 200 Xg for 5 minute, then cells were resuspended in 1 ml Hank's buffered salt solution (HBSS) and then cells were transferred into 10 ml of ice-cold 70 % ethanol; stored at -20 ºC for 24 hrs or longer. The fixed cells were spun down again at 800 xg for 5 minutes and ethanol was removed thoroughly. The cell pellet was resuspended in 40 µl phosphate-citrate buffer (consisting of 192 parts of 0.2 M Na_2HPO_4 and 8 parts of 0.1 M citric acid pH 7.8) and then incubated at room temperature for at least 30 min. Cell lysate was span down at 1000 xg for 5 minutes. The supernatant was transferred to new tubes and 3 µl of 0.25 % Nonide NP-40 (in water) and 3 µl of RNase (1 mg/ml in water) was added to the samples and incubated for 30 min at 37º C. 3 µl of proteinase K (1mg/ml) was added to the samples and was incubated for another 30 minutes at 37 ºC. Finally, 12 µl of loading buffer (0.25 % bromophenol blue, 30 % glycerol) was added to the samples and the mixture was loaded on a 1.5 % agarose gel containing ethidium bromide. Electrophoresis was run at 100 V, DNA were taken under a UV trans-illuminatior.

Determination of ROS

ROS levels were assayed using the Image-iT™ LIVE Green Reactive Oxygen Species Detection Kit (Invitrogen). Briefly, cells were plated onto a 'Fluorodish', a glass bottom 35-mm cell culture dish designed for microscopy (World Precision Instruments, Sarasota, FL), 24 hr. After the indicated treatments, the cells were gently washed with warm Hank's balanced salt solution (HBSS) and incubated with carboxy-H_2DCFDA (10 µM × 60 min at 37° C) to detect ROS. The cells were washed with HBSS; incubated with DCF (1 µM × 5 min at 37° C) to stain the nuclei; washed; and visualized using an Olympus microscope. The fluorescence from the dyes was observed using a standard fluorescein filter set, rhodamine filters and a filter DAPI set, respectively. Green ROS fluorescence was quantitated using Metamorf image analysis software (Molecular Devices, Downington, PA) based on 100 cells and expressed as means ± SEMs (arbitrary units) of three independent experiments.

RESULTS

Phytochemical screening of methanol extract of *I. aspalathoides*

The preliminary phytochemical screening of *I. aspalathoides* has revealed the presence of steroids, saponins, flavonoids, terpenoids, phenolic compound, anthraquinones, tannins and reducing sugar were present. Alkaloids were completely absent which are given in Table - 1.

Separation of phytochemical compounds by thin layer chromatography

The R_f value were calculated by measuring the distance travelled by the solvent and the solute and the corresponding value of the each compounds are 0.636, 0.650, 0.566, 0.533 and 0.550 respectively. Results are given in Table 2.

Antimicrobial activity of the root extract:

The extracts from *I. aspalathoides* plant displayed antibacterial and antifungal activities with the centimeters (cm) of inhibition zone ranging between 0.6 and 1.8 cm. All three concentrations showed varying degrees of inhibition on the tested microorganisms. The naked eye judgment method is used to determine the end point of inhibition at the edge where the growth starts. The methanolic root extract inhibited the growth of all bacterial and fungal strains tested. The methanolic root extract showed highest inhibition zone against *K. pnemoniae* (1.8 cm) (Table 3.a, Fig.1). The methanolic root extract showed highest inhibition zone against fungal strain *A. fumigates* (1.6 cm) (Table 3.b, Fig. 2).

Radical scavenging activity (RSA) of *Indigofera aspalathoides* extracts (DPPH ASSAY)

From the dose dependent response curve of DPPH radical scavenging activity of methanolic extract of *I. aspalathoides*, it was observed that the methanolic extract had higher scavenging activity (Table 4.a). At a concentration of 20 µg/ml, the scavenging activity of methanol extract reached 54.3 ±2.2, which was comparable to that of standard chemical. The methanol root extract of *I. aspalathoides* showed excellent antioxidant and free radical scavenging activity.

Hydroxyl radical scavenging activity

The hydroxyl radical is one of representative reactive oxygen species generated in the body. These radicals are produced through various biological reactions; one of the common reactions is the Iron (II)-based Fenton reaction. Hydroxyl radical scavenging activity of methanolic root extract and standard is presented in Table 4.b. The radical scavenging capacity may be attributed to phenolic compounds in methanol extract with the ability to accept electrons, which can combine with free radical competitively to decrease hydroxyl radical. IC_{50} value was found be 32.5µg/mL against the hydroxyl radical scavenging activity.

Cytotoxic effect of methanolic extract of *I. aspalathiods* treated NCI h460 lung cancer cells

The cytotoxic effects of methanolic root extract of *I. aspalathiods* were examined on cultured NCI h460 human lung cancer cells by exposing cells at various concentrations for 24 and 48 hr. The reduced MTT-formazan was dissolved in DMSO and the absorbance was read in 96 well plate reader. The graphs were plotted as % of inhibition (absorbance at Y-axis) against the concentration of the drug (X-axis). The IC_{50} concentration was determined as the drug concentration that is required to reduce the absorbance to half that of the control. The IC_{50} value in respect of the methanolic extract on the cell line was shown in the Fig.3. The extract brought about death of the cancer cells in a dose- and duration-dependent manner.

Table.1. Phytochemical Screening in Methanolic root extract of *Indigofera aspalathoides*

S. No	Phytochemicals	Result
1	Detection of Alkaloids	-
2	Detection of Phenolic compound	+
3	Detection of Anthraquinones	+
4	Detection of Terpenoids	+
5	Detection of Flavonoids	+
6	Detection of Tannins	+
7	Detection of Reducing sugars	+
8	Detection of Saponins	+
9	Detection of Steroids	+

+ Present - Absent

Table.2. Compound identified from the Root extract of *Indigofera aspalathiods* by TLC

S.No	Phytochemical compound	Color of the spot in the TLC plate	RF value
1	Flavonoid	Fluorescence	0.636
2	Saponins	Yellow	0.650
3	Tannin	Green	0.566
4	Steroid/Terpenoid	Greenish black	0.533
5	Phenol	Dark blue	0.550

RF: Retention factor (It is the distance traveled by the sample or analyte divided by distance traveled by the solvent front in chromatography)

Table 3.a.Bacterial Inhibition zones (cm) of Methanolic root extract of *Indigofera aspalathoides*

S.No	Bacterial strains	50 µl	75 µl	100 µl	Positive control
1	*Salmonella typhi*	0.6	1.0	1.1	1.3
2	*Staphylococcus aureus*	0.5	0.9	1.2	1.6
3	*Klebsiella pneumonia*	0.6	1.3	**1.8**	1.7
4	*Escherichia coli*	0.2	0.6	0.9	1.2
5	*Streptococcus pyogenes*	0.5	0.7	1.0	1.3
6	*Pseudomonas aeruginosa*	0.5	0.9	1.0	1.4

Positive control - Streptomycin – (10mg/ml)

Table. 3.b. Fungal Inhibition zones (cm) of Methanolic root extract of *Indigofera aspalathoides*

S.No	Fungal strains	50 µl	75 µl	100 µl	Positive control
1	*Aspergillus flavus*	0.5	1.0	1.2	1.5
2	*Aspergillus fumigates*	0.8	1.3	**1.6**	1.7
3	*Aspergillus niger*	0.6	1.2	1.5	1.7
4	*Candida albicans*	0.4	0.8	1.1	1.3

Positive control - AmphotericinB – (10mg/ml),

Morphological changes in the cells after methanolic extract of *I. aspalathiods* treatment

Acridine orange (AO) - Ethidium bromide (EB) and Hoechst staining were done on NCI h460 cells treated with respective IC_{50} concentration for 24 and 48 hr. The control cells appeared green and blue fluorescent, respectively, whereas the extract treated cells revealed apoptotic morphologies. Staining NCI h460 cells treated with the methanolic extract showed higher percentage of apoptotic death (red fluorescence) and lower percentage of necrotic death. Staining of the cells revealed membrane blebbling, presence of apoptotic bodies, marginalization of chromatin and innumerable micronuclei in cells, thus, showing apoptotic features (Fig. 4). Hoechst staining for cells treated with the methanolic extracts revealed more cells with abnormal nuclear morphologies such as chromatin condensation and nuclear fragmentation (Fig. 5).

Induction of DNA fragmentation by methanolic extract of *I. aspalathiods*

DNA fragmentation is an obvious characteristic in cells entering apoptosis, it was reported that methanolic extract of *I. aspalathiods* induced DNA fragmentation on human lung cancer cells NCI h 460. To assess whether methanolic extract of *I. aspalathiods* has the DNA fragmentation activity effects, we investigated the capability of methanolic extract on the induction of DNA fragmentation in both 24 and 48 hours treatment. Agarose gel electrophoresis results as shown in (Fig. 6**);** DNA fragmentation appeared in NCI h460 cells and no fragmentation in untreated cells.

Determination of ROS

Fluorometric intracellular ROS measurement by DCFH-DA method was performed in methanolic extract of *I. aspalathiods* treated and untreated cells. Recent study showed that ROS could induce autophagy formation in certain types of cancer cells. Highest ROS accumulation was observed from methanolic extract of *I. aspalathiods* treated NCI h 460 cells at 24 and 48 hrs (Fig. 7).

Table .4. Antioxidant activity of Root extract of *Indigofera aspalathiods*

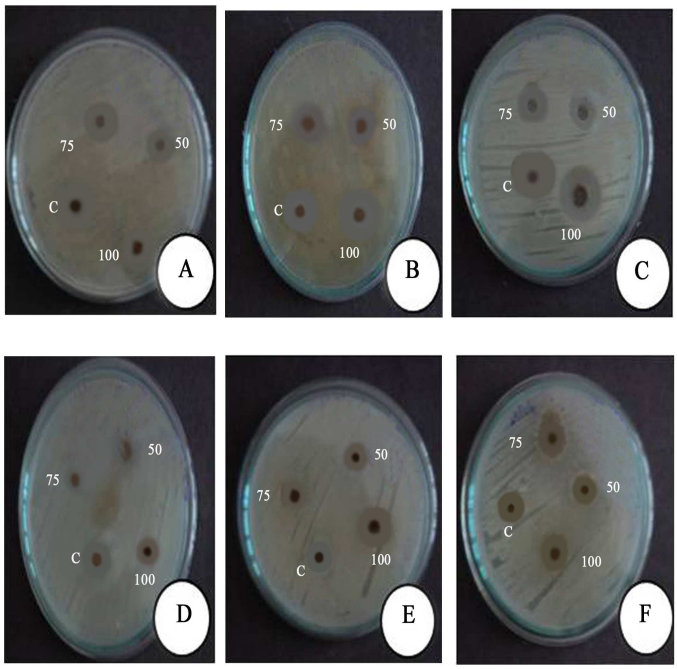
Table. 4.a. DPPH radical scavenging Activity

S. No	Concentration of Fraction used (µg/mL)	Methanol fraction % of inhibition	Standard % of inhibition
1	1	22.3 ± 1.4	30.1 ±2.0
2	2	30.2 ± 2.1	35 .3±1.8
3	5	35.6 ± 1.6	48 .4±1.1
4	10	41.9 ± 1.8	52 .2±1.5
5	15	48.4 ± 1.9	59 .0±0.6
6	20	54.3 ±2.2	67 .7±0.9
	IC 50	**18.1 µg/mL**	**8.8 µg/mL**

Table.4.b. Hydroxyl radical scavenging Activity

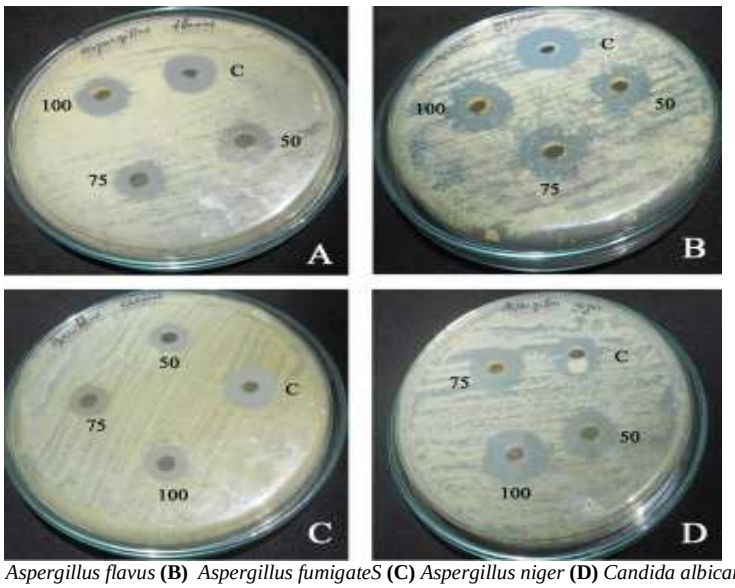
S. No	Concentration of Fraction used in µg/mL	Methanol fraction % of inhibition	Standard % of inhibition
1	10	34.2 ±1.8	7.1 ±0.5
2	20	39.0 ±1.5	12.2±0.91
3	30	45.1 ±1.4	18.6 ±2.1
4	40	56.3 ±1.3	24.3 ±1.7
5	50	64.7 ±1.2	33.8 ±1.6
6	60	78.4 ±1.1	43.4±0.6
7	70	84.5 ±0.5	54.0 ±1.7
	IC 50	**32.5µg/mL**	**67.8 µg/mL**

Fig.1. Antimicrobial activity of *Indigofera aspalathoides* Bacterial pathogens



(A)Salmonella typhi **(B)** *Staphylococcus aureus* **(C)** *Klebsiella pneumoniae* **(D)** *Escherichia coli* **(E)** *Streptococcus pyogenes*(**F**) *Pseudomonas aeruginosa*

Fig.2. Antimicrobial activity of *Indigofera aspalathoides* Fungal Pathogens



(A) Aspergillus flavus **(B)** *Aspergillus fumigateS* **(C)** *Aspergillus niger* **(D)** *Candida albicans.*

Fig.3. Cytotoxicity of methanolic root extract of *Indigofera aspalathoides*

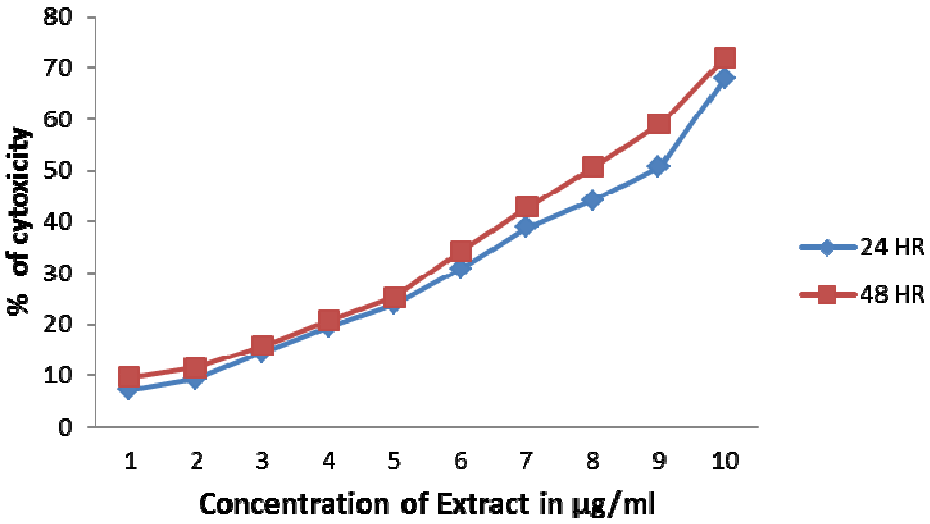
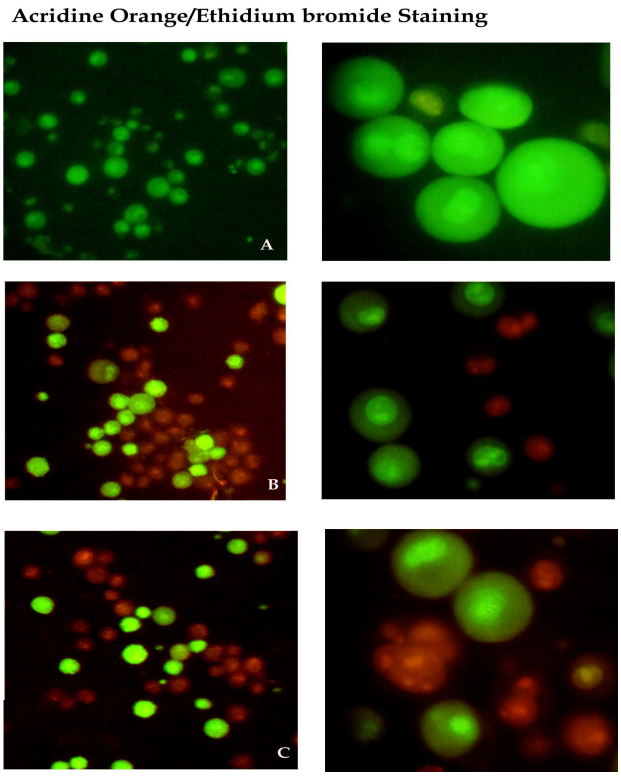


Fig.4. Morphological changes induced by methanolic root extract of *Indigofera aspalathoides*



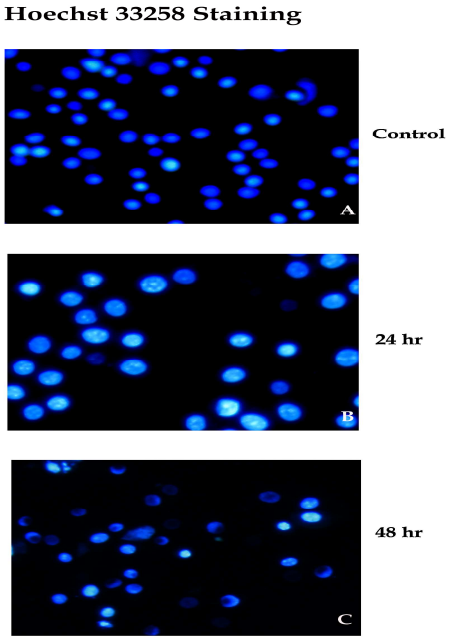
A – Control, B – 24 Hr treatment with Methanolic root extract, C - 48 Hr treatment with Methanolic root extract

Percentages of Cytotoxicity of methanolic root extract of *Indigofera aspalathoides* in NCI h460 cells

Conc. of the extract	% of Cytotoxicity	
	24 h	48 h
1	7.054	9.57
2	9.17	11.42
3	14.301	15.74
4	19.315	20.58
5	23.832	25.12
6	30.71	34.17
7	38.812	42.75
8	44.021	**50.63(IC_{50})**
9	**50.62(IC_{50})**	58.94
10	67.931	71.91

IC_{50} - Concentration required to kill 50% of the cells

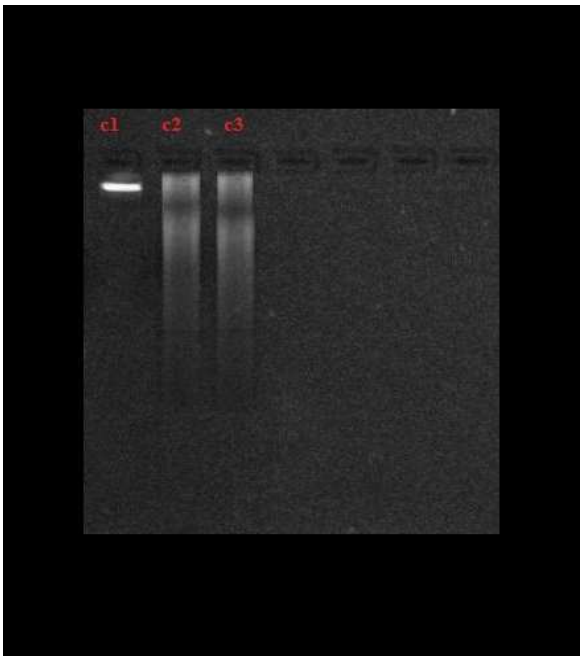
Fig.5. DNA damage induced by methanolic root extract of *Indigofera aspalathoides*



Rate of ROS production in methanolic root extract of *Indiofera aspalathiods* in NCI h460 cells

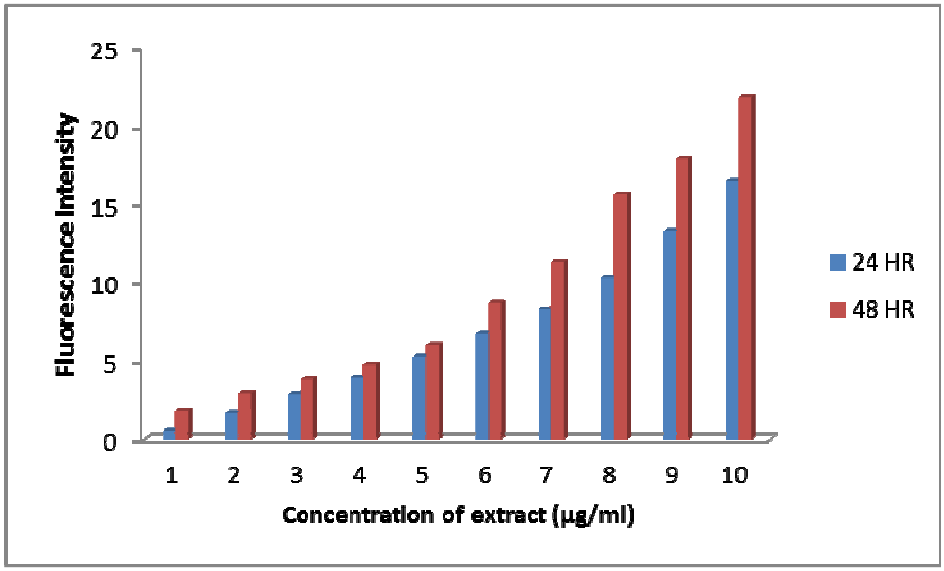
Conc. of extract (µg/ml)	Rate of ROS Production	
	24 hr	48 hr
1	0.521	1.87
2	1.72	2.94
3	2.89	3.81
4	3.9	4.79
5	5.31	6.01
6	6.74	8.73
7	8.31	11.33
8	10.36	15.63
9	13.34	17.92
10	16.52	21.83

Fig.6. DNA Fragmentation induction in NCI h460 cells by methanolic root extract



C1 – Control, C2 – DNA fragmentation at 24 hr, C3 - DNA fragmentation at 48 hr

Fig.7. Determination of Intracellular ROS production



DISCUSSION

Preliminary phytochemical screening of the methanolic root extract of *indigofera aspalathoides* revealed that the presence of flavonoid, phenols, tannins, saponins, steroidal terpenes and quinines. These compounds may have remarkable antimicrobial, antioxidant and cytotoxic effects. Most plant extracts were found to have major bioactive compounds to be present in different solvent extract. Each bioactive compound has its specific activity for example, alkaloids have been used treat disease like malaria, pain killers and managing heart diseases (Oomah, 2003). Phytochemical study of the crude powder of 53 plants showed the presence of alkaloids, tannins, cardiac glycosides steroids and saponins. The methanol extract of *Mangifera indica* showed the highest total phenolic content while

acetone extract of *Aristolohia bracteolate* showed highest flavonoid content than other plant extracts (Vaghasiya *et al*., 2011).

The methanolic root extract of *indigofera aspalathoides* inhibited the growth of bacterial and fungal pathogens. The maximum zone of inhibition was measured in *Klebsiella pneumonia* (1.8 cm). when compare to bacterial pathogens the zone of inhibition was minimal in fungal pathogens such as *Aspergillus fumigates* (1.6 cm), *Aspergillus niger* (0.6 cm), *Aspergillus flavus* (0.5 cm) *and Candida albicans* (0.4 cm). This is similar to the finding of Akroun *et al*., (2009) who reported the methanol to be the best solvent for extraction of most plant active principle such antibacterial and anticancer. The anti microbial activity of the methanolic extract appears to have a broad spectra of activity on both gram positive and gram negative bacteria. From the above said reference the present study indicates that methanol is the appropriate solvent to extract bioactive compounds.

The DPPH assay is based on the measurement of the relative inhibition of the extract, test at various concentrations. Chemicals which are able to change the colour of DPPH free radical from purple to yellow can be considered as antioxidant (Hinneburg *et al*., 2006). In the present study, the antioxidant activity of methanol leaf extract of *Indigofera aspalathoides* was observed at various concentrations from 1-20 µg/ml. The value of 50% inhibition concentration (IC_{50}) was found at 18.1 µg/mL. The result showed that the root extract has strong antioxidant than the standard ascorbic acid. In earlier study, the root extract of *Indigofera* species showed good antioxidant activity. Among the species, *Indigofera tinctoria* showed the good antioxidant activity with an IC_{50} 3.79 at 0.08 µg/ml. Similarly the aqueous soluble fraction of the methanol extract of *Centella asiatica* revealed potent antioxidant activity with the IC_{50} value at 7µg/ml on the other hand, the carbon tetra chloride n-hexane soluble fraction showed moderate antioxidant activity with the IC_{50} value of 40 and 298 µg/ml respectively (Obayed ullah *et al*., 2009).

In the present investigation, the methanolic root extract of *Indigofera aspalathoides showed potent cytotoxic activity against human lung cancer cell line (*NCI-H460*)*.The percentage of cell viability, lowest cell viability 50% was showed 8 and 9µg/ml for 24 and 48 hr respectively. On the morphology of the cells, cell membrane cleavage, shrinkage, nuclear condensation, apoptotic bodies' formations were highly found in methanolic root extract treated cells. Recent study on *Indigofera tinctoria*, the flavonoidal portion had the cytotoxicity on lung cancer cell line (Kameswaren and Ramanibai, 2008). Rahman et al., (2008) reported that among the preliminary cytotoxicity screening of some medicinal plants of Bangladesh, *Indigofera tinctoria* showed highly lethal to brine shrimp naupli. Hence, the plant *Indigofera aspalathoides* has strong antioxidant and cytotoxic properties, further evaluation on the phyconstituents of the plant will provide novel therapeutic interventions.

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