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Pharmacological Investigation of *Lawsonia Inermis* L. Seed Extracts: An *Invitro* Study

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ABSTRACT
Lawsoniainermis L. belongs to branched glabrous shrub. They are mainly cultivated for its leaves. The seeds, bark, roots, flowers also are used in traditional medicine. In the present study the hexane and methanolic extracts of *L.inermis* seeds were analysed for phytochemical analysis, antioxidant, antibacterial and anticancer activites. The preliminary phytochemical analysis in hexane and methanolic extracts showed the presence of compounds such as carbohydrates, proteins, flavonoids, tannins, phenolic compounds, alkaloids, terpenoids, and fatty acids. The free radical scavenging activity using DPPH assay showed that the methanolic extract possessed higher antioxidant potential. The antibacterial activity assessed using the seed extract showed activity against gram negative bacteria and the zone of inhibition was prominently seen in the methanolic seed extract. The anticancer activity using MCF 7 cell line exhibited promising results and the percentage of cell viability decreased after treated with methanolic seed extract. Thus the study proves that *L.inermis* seeds have phenomenal medicinal values to be explored in the future.
Keywords: *Lawsoniainermis*, Phytochemicals, antioxidant, antibacterial, anticancer.

1. INTRODUCTION
Plants contain a wide range of chemical compounds that can be used to cure chronic diseases **(Duraipandiyan, 2006)**. The information and benefits of herbal drugs are abundant in our ancient literature of Ayurvedic, Siddha, Unani and Chinese medicine. Around 80 % population of developing countries are being unable to afford pharmaceutical drugs and rely on traditional medicines, to sustain their primary health care needs **(Bandyopadhyay, 2002)**. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare **(Cragg*et al*., 1997)** because of their wide biological and medicinal activities, higher safety margins and lesser costs.

Especially in India 80% of the population depends on plants for their therapeutic purposes **(Hiren*et al* ., 2013)** 60% population use plant based medicine to battle certain ailments and 40% humans rely on herbs that are employed in pharmaceutical industries.

World Health Organization has suggested the use of phytomedicine for curing various ailments. The necessity of using the plant as a medicine is huge due to the wide diversity of

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plants around the world. Cultural and geographical **(Samuelsson, 2004)** factors also facilitate the treatment for various diseases with plants in different formulations like crude plant extracts. The ancient history of India describes its diverse uses and also plays a vital role in the ayurvedic or natural herbal medicines (**Lavhate 2007**). Most of the countries are exploring the herbal plants in search for new drugs, with curative properties to prevent various diseases. The compounds from medicinal plants are patented in many developing countries.

L. inermis commonly known as henna or mehendi is abundantly available in tropical and subtropical areas. Henna leaves, flowers, seeds, stem, bark and roots are used in native medicine to treat a variety of ailments (**Chetty, 2008**) for rheumatoid arthritis, headache, ulcers, diarrheoa, leprosy, fever, leucorrhoea, diabetes, cardiac disease and hepatoprotective.

Phytochemicals are naturally found in plants which possess health benefits. These are known as secondary metabolites (**Kabera*et al*., 2014)** and have modified synthetic pathways from primary metabolites.

Dietary intake of plants rich in phytochemicals helps in protection of humans. These dietary phytochemicals are **(Saxena*et al*., 2013)** mostly present in fruits, vegetables, seeds, herbs and spices. Medicinal plants due to their antioxidant potential show decreased toxicity comparing with the chemically formulated drugs **(Kooti, 2014; Grzanna, 2005).**

Cancer is being one of the **(WHO, 2013)** causes for death all over the world**.** As reported by WHO there might be 21.4 million cases of people affected by cancer and 13.2 million mortality due to cancer by 2030 **(ACS, 2011).** There is an urgent need for the prevention and treatment for this deadly disease. As indicated above medicinal plants play a significant role in the synthesis of a new drug. The present study is focused on the pharmacological properties of *L.inermis* seeds.

2. MATERIAL AND METHODS

2.1 Plant Collection:

The fresh seeds of *L.inermis* were harvested during the months of November and December. The seeds were washed thoroughly and allowed to dry. The seeds after drying were ground coarsely using electrical blender. The powdered sample was subjected to extraction.

2.2 Extraction of Plant Material:

The powdered sample was soaked in hexane and methanol solvents for about 48h. The extract was then filtered using Whatmann's filter paper. The obtained extract after filtration was concentrated using rotary evaporator. The extracts were stored in air tight bottles for further experiments.

2.3 Qualitative Phytochemical Analysis:

The hexane and methanol extracts were tested for the presence of secondary metabolites. Standard procedures were used for identifying the phytoconstituents**(Harborne, 1973)**.

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2.3.1 Test for Alkaloids

Dragendroff's test:

2ml of the extract was taken in a 25ml test tube and about 2ml of the dragendorff's reagent was added to the extract. A prominent yellow colour precipitate indicates the presence of alkaloids in the extract.

2.3.2 Test for Carbohydrates

Fehlings test:

1ml of the filtrate was taken in a test tube and left to boil in a water bath for a few minutes.1ml of Fehling's solution A & B was added to the boiled filtrate. A red precipitate shows the presence of carbohydrates.

2.3.3 Test for Glycosides

50µl of the extract was hydrolysed with a few drops of concentrated HCl and the mixture was boiled in a water bath for a few minutes and then filtered.

Borntragers test:

2ml of the filtrate was then taken and 3ml of choloform was added and shaken, the chloroform layer separates and 10% ammonia solution was added. Pink colour indicates presence of glycosides.

2.3.4 Test for Proteins and Amino acids

100mg of the extract was taken and 10ml of distilled water was added and the mixture was filtered through a Whatmann's No.1 filter paper.

Ninhydrin test:

2 drops of the Ninhydrin reagent was taken in a test tube and 2ml of the plant extract was added. Presence of proteins and amino acids indicates purple colour.

2.3.5 Test for Phenolic Compounds

Ferric chloride test:

To 50mg of the extract, 5ml of distilled water was added and a few drops of neutral 5% ferric chloride was added to the mixture. A dark greenish colour indicates presence of phenolic compounds.

2.3.6 Test for Flavonoids

5ml of the extract is taken in a test tube and 2ml of ammonia solution is added to the extract. The change in the mixture to yellow indicates the presence of flavonoids.

2.3.7 Test for Terpenoids

Salkowski test:

5 ml of the extract is taken in a test tube and 2ml of chloroform is added to the extract. To this mixture add a little or few drops of concentrated sulphuric acid. The formation of a reddish brown layer at the interface of the two liquids shows the presence of terpenoids.

2.3.8 Test for Saponins

Foam test:

To 1mg of the plant extract add 2ml of distilled water and shake the mixture well. The persistent froth on the mixture confirms the presence of saponins.

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2.3.9 Test for Steroids

Liebermann-Burchardt test:

0.2ml of chloroform is added to the extract in a test tube and then a few drops of acetic acid were poured and concentrated sulphuric acid is added to the mixture. Appearance of a mixture of blue and green colour indicates the presence of steroids.

2.3.10 Test for tannins

Braemer's test:

To 1ml of the extract add 2ml of distilled water and then 2 drops of ferric chloride is added. The change in the colour of the solution from green to blue green indicates the presence of tannins.

2.4 Antioxidant Activity

The DPPH free radical scavenging activity was assessed in the seeds of hexane and methanolic extracts. 0.1mM of DPPH solution in methanol was prepared. 1ml of DPPH was added to 3ml of the plant extracts (Hexane, Methanol) in different concentrations of 20,40,60,80,100 µg/ml. The above mixture was **(Ahmad *et al*., 2013)** shaken and allowed to stand for about 30min. The absorbance was taken at 517nm using UV spectrophotometer. Here the reference standard used was ascorbic acid **(Rajesh and Natvar, 2011)**. The free radical scavenging activity was calculated using the formula as follow

$$\textbf{Percent Inhibition} = A_0\text{-}A1/A_0 \times 100$$

Where A_0 is the absorbance of the control and A1 is the absorbance of the standard or plant extract **(Achola and Munenge, 1998)**.

2.5 Antimicrobial Activity

The antibacterial activity was tested using agar diffusion method **(Kokate, 2000)**. The solvents that were initially used to dissolve samples were employed as negative controls. The inhibition zones were measured and compared with that of the standard drug amoxicillin. Each of the extract was serially diluted with DMSO solvent to give 2mg/ml, 1 mg/ml,0.5mg/ml and 0.25mg/ml solutions. The concentration of amoxicillin was fixed at 1mg/ml. The prepared samples were tested for antimicrobial activity against three gram negative bacteria namely ***P.aureginosa***, ***S. aureus***, ***Enterobacter*** on nutrient agar plate using disc diffusion method. Whatmann's No.1 filter paper discs were impregnated with hexane and methanol extracts of *L.inermis* seed. The plates were incubated at 37^0C for 24h.The antimicrobial activity was evaluated by measuring the zone of inhibition against the tested bacteria. Each of the tests carried out were done in triplicates.

2.6 Cell Culture

For this study MCF -7 Breast Cancer Cell Line were used. The cells were grown in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% FBS and 1% antibiotic mixture of penicillin /streptomycin. The cells were maintained in a with humidified atmosphere with 5% CO_2 at 32^0C in a steri-cycle il160 incubator and checked at regularly.

2.6.1 Antiproliferative MTT assay

The antiproliferative activity was carried out according the method described by **(Szabo, 2016)**. The cell were plated at a density of 5000 cells /well using 96 well plates and was incubated with selected concentrations of the selected extract. After 72h of incubation, 5mg/mL MTT solution was added and incubated for another 4h. The absorbance of the

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precipitated formazan crystals dissolved in DMSO was measured **(Mosmann, 1983)** with the microplate reader at 545nm. Wells with cells incubated with medium and DMSO were taken as control.

3. RESULTS AND DISCUSSION

Extraction is the process of separating active biological compounds using solvents. During the process of extraction the solvents diffuse into plant material and solubilise the compounds with similar polarity. Earlier studies on the extraction reveal that methanol solvent can extract most of the active compounds **(Cowan, 1999)** compared to the other solvents. Most of the compounds extracted using methanol solvent was polar in nature such as anthocyanins, flavones, quassinoids, polyphenols etc. Chloroform solvent extract compounds such as terpenoids and flavonoids whereas hexane extracts lipids. The preliminary phytochemical analysis of the hexane and methanol extracts of the seeds of *L.inermis*revealed the presence of alkaloids, flavonoids and phenols in both hexane and methanol extracts. Whereas the presence of tannins, steroids, terpenoids, tannins, saponins and glycosides were found to be present only in the methanolic extract of seeds.

Phytochemicals are known for curative activity against diseases like ulcer, swollen liver, malaria, diarrhoea. The medicinal plant contains various phytoconstituents that are of valuable resources for therapeutics. alkaloids, **(Singh *et al*., 2014)** flavonoids and glycosides have biological effects such as anti-inflammatory, anti-allergic, anti-diabetic, antioxidant, antiviral, anticancer, antileprosy, and antimicrobial. Alkaloids are natural chemicals which contain basic nitrogen. They are rich in **(Rhoades, 1979**) pharmacological effects and are used in recreational drugs.

Flavonoids increase the effect of vitamin C and play a vital role as antioxidants. The flavonoids are active against tumour, virus, **(Yamunadevi*et al*., 2011)** liver toxins and microbes. Terpenoids present in the plants are used for aromatic properties and are widely used in herbal medicines.

Earlier reports on phenols reveal that they are used as anti-tumour agents and possess antioxidants **(Robak and Gryglewski, 1988).**

The principle behind free radical scavenging activity is based on one electron reduction (i,e) it represents the free radical reducing activity of antioxidants. Ascorbic acid was used as positive control. The findings on the antioxidant activity show that *L.inermis* seed extract possess antioxidant activity. The DPPH assay revealed the highest antioxidant activity in the methanol extract compared to the hexane extract. The lowest IC_{50} was detected for methanolic extract followed by hexane. The lower IC_{50} value has a higher antioxidant potential. The earlier reports on antioxidant **(Kabra*et al*., 2019)** results were highly related with total phenolic and total flavonoid content. Antioxidants play a vital role by balancing and breaking free radicals thus cease oxidative stress related diseases **(Maeura*et al*., 1984).**

The antibacterial effect of *L.inermis*seed extracts are presented in *P. aureginosa.* Amoxicillin was the standard drug that used to evaluate the antibacterial activity in the present study. The methanolic extract was found to be active against ***P. aureginosa,***

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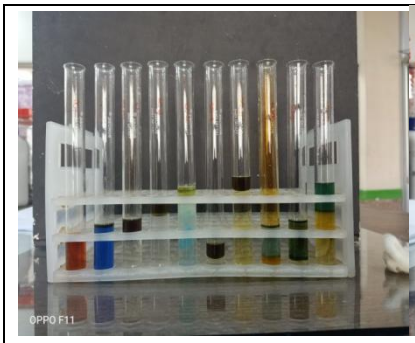
Enterobacter, S. aureus. The extract that exhibited the highest activity was selected for anticancer activity.

The crude methanolic extract of *L. inermis* seed was investigated on the growth of MCF 7 cell line using MTT assay. The maximum percentage of viability was observed at the concentration of 100 (µg/mL) that was **(Singh and Luqman, 2014)** 23.43%. The minimum percentage inhibition was observed at 20 (µg/mL) concentrations that were 94.04 %. The morphological observation of control and treated cells are given in. Previous report on *L.inermis* leaf extract showed IC_{50} value of 24. 85 (µg/mL).

4. RESULTS

QUALITATIVE PHYTOCHEMICAL ANALYSIS



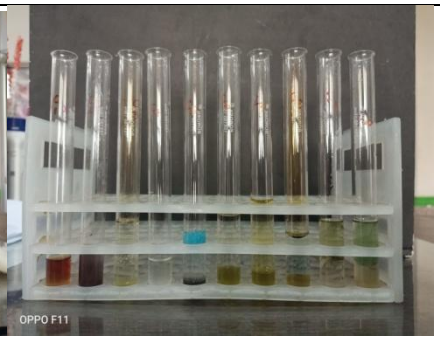


Fig:1 Phytochemical analysis of HexaneFig:2 Phytochemical analysis of Methanol

Phytoconstituents	**Name of the test**	**Hexane**	**Methanol**
Alkaloids	Dragendroff's test	+	++
Carbohydrates	Fehling's test	–	–
Glycosides	Borntrager's test	–	+
Proteins/ amino acids	Biuret and Ninhydrin test	–	–
Phenol	Ferric Chloride	+	++
Flavonoids	Alkaline reagent test	+	++
Terpenoid	Salkowski's test	–	+

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Saponin	Foam test	–	+
Steroids	Liebermann-Burchardt test	–	+
Tannins	Braemer's test	–	+

Table1: Phytochemical Analysis of Hexane and Methanolic Extracts

Antioxidant Activity using DPPH Assay

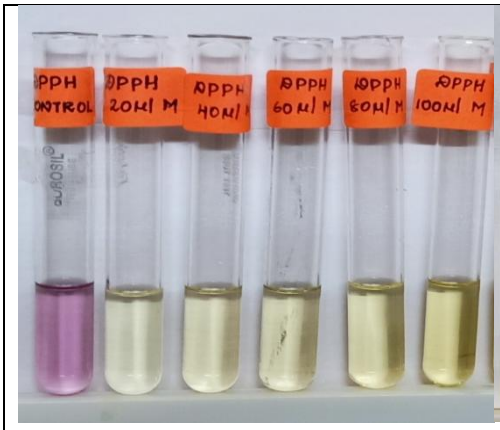

Fig:3 Antioxidant activity of Hexane

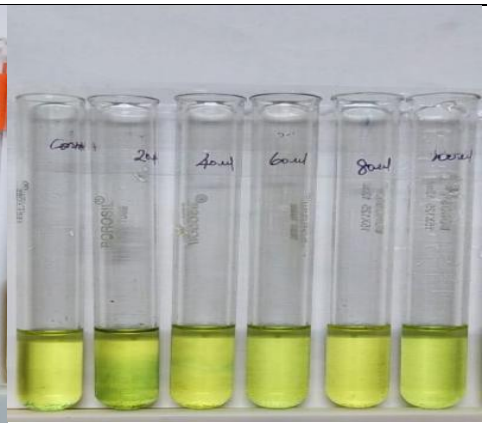

Fig:4 Antioxidant activity of Methanol

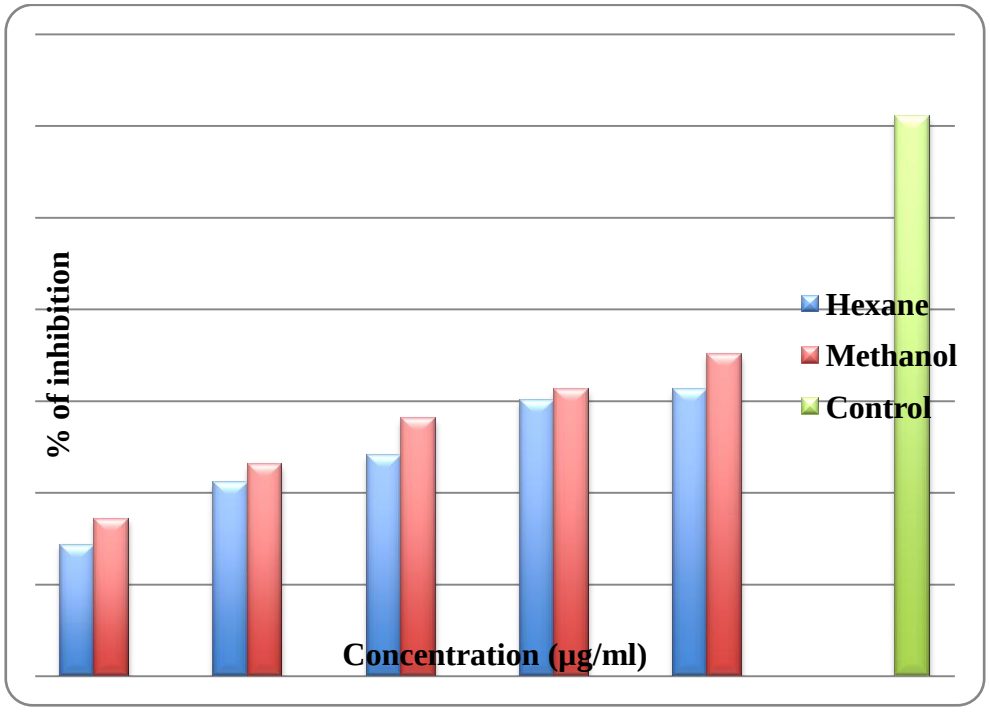

Fig:5 DPPH activity of Hexane and Methanolic Extracts of *L.inermis* seeds

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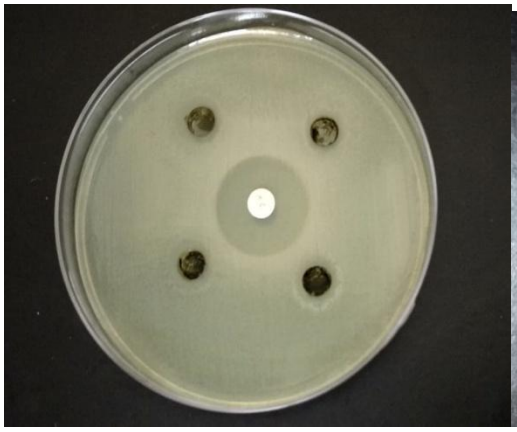

Fig:6*P.aureginosa*

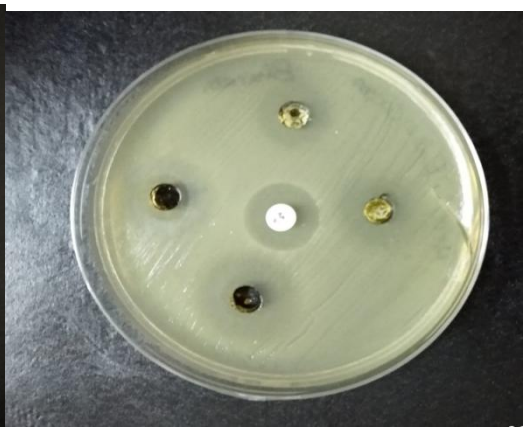

Fig:7*Enterobacter*

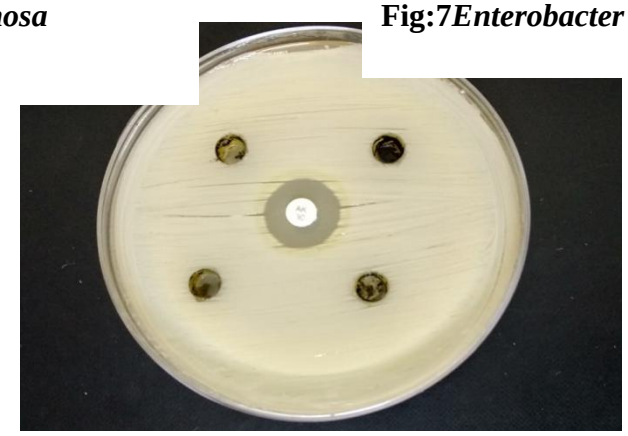

Fig:8*S.aureus*

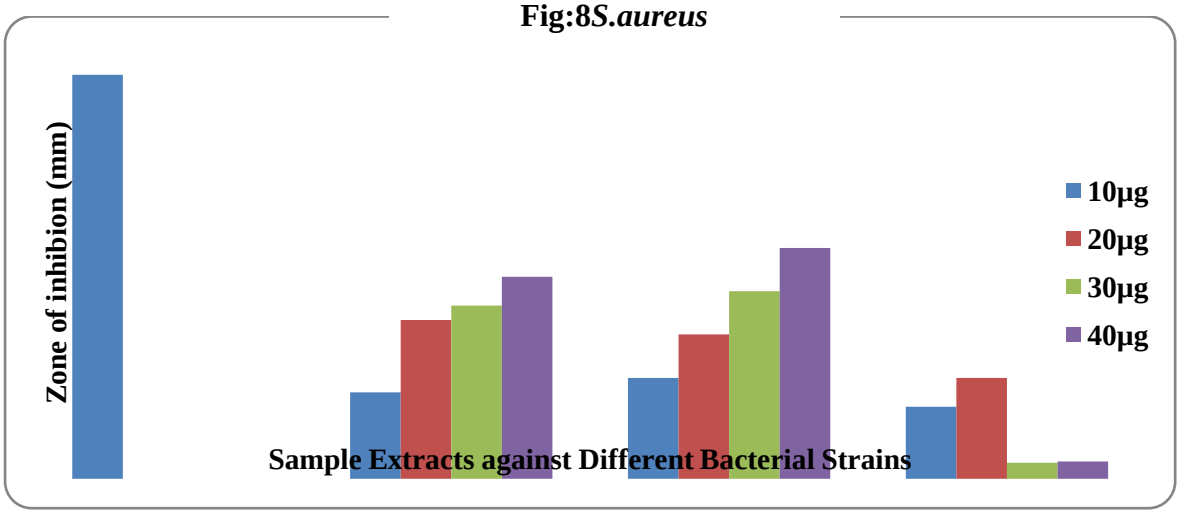

Fig:9 Antibacterial activity of Hexane Extract

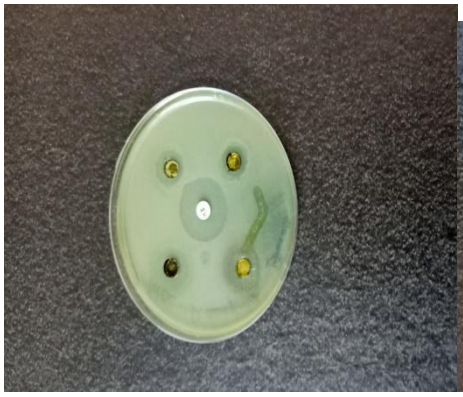

Fig:10*P.aureginosa*

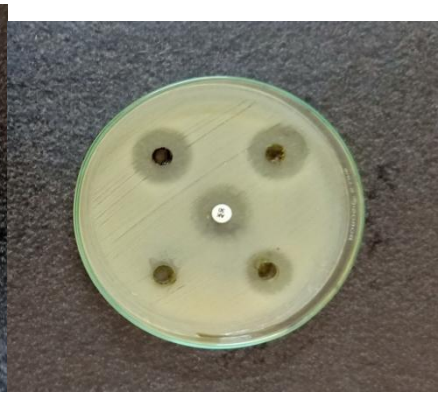

Fig:11*Enterobacter*

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Fig:12*S.aureus*

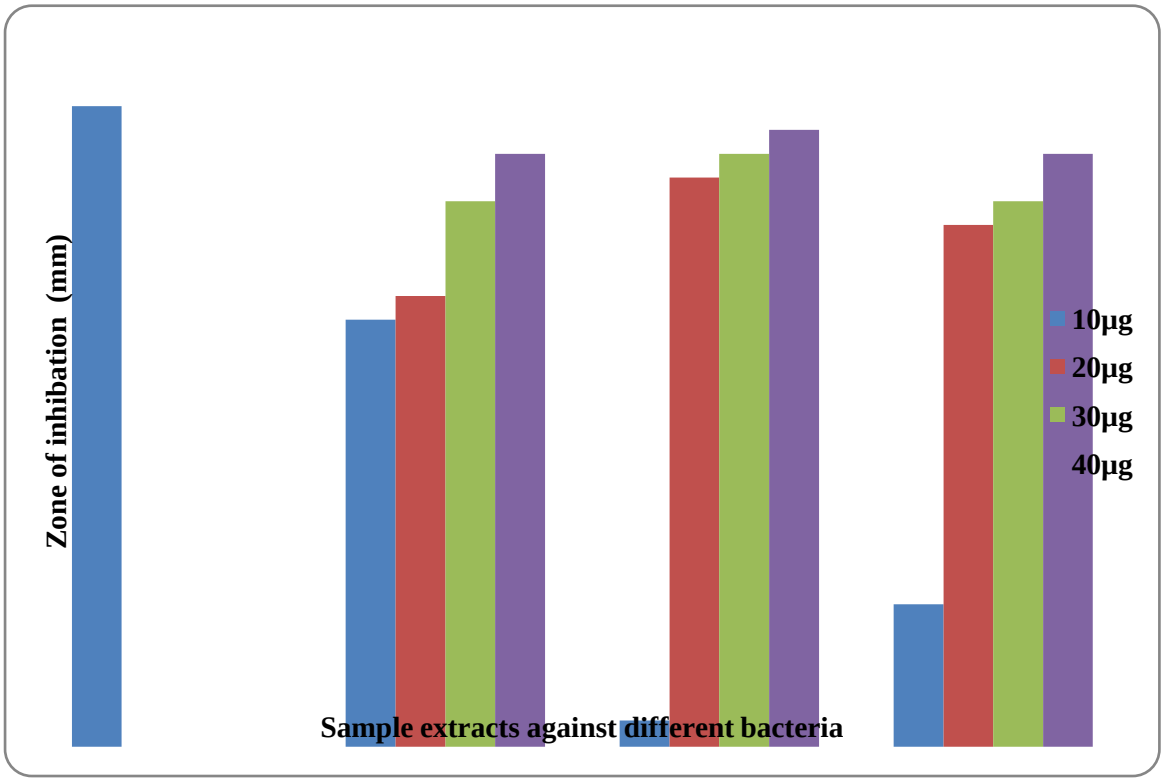

Fig:13 Antibacterial activity of Methanolic Extract

ANTICANCER ACTIVITY

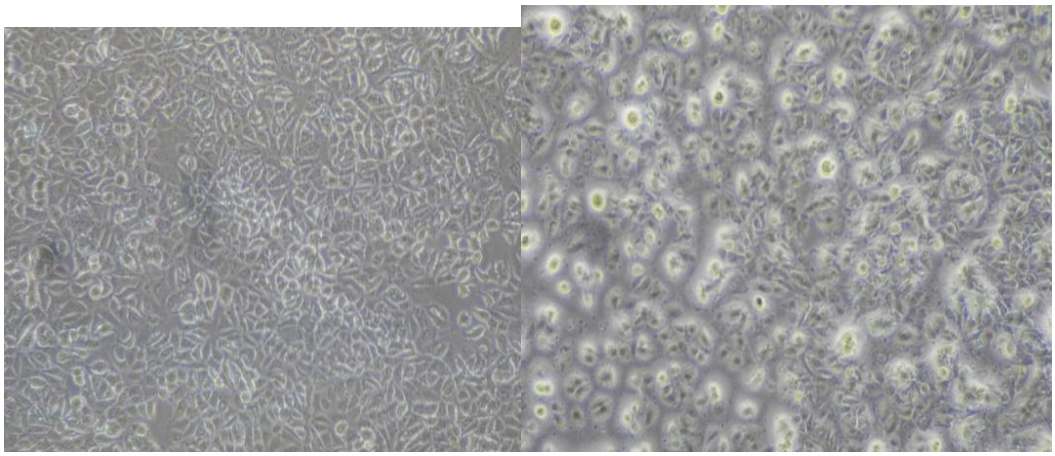

Fig:14Control Cells

Fig:15Treated cells with *L.inermis* seed MethanolicExtract at 100 (µg/ml)

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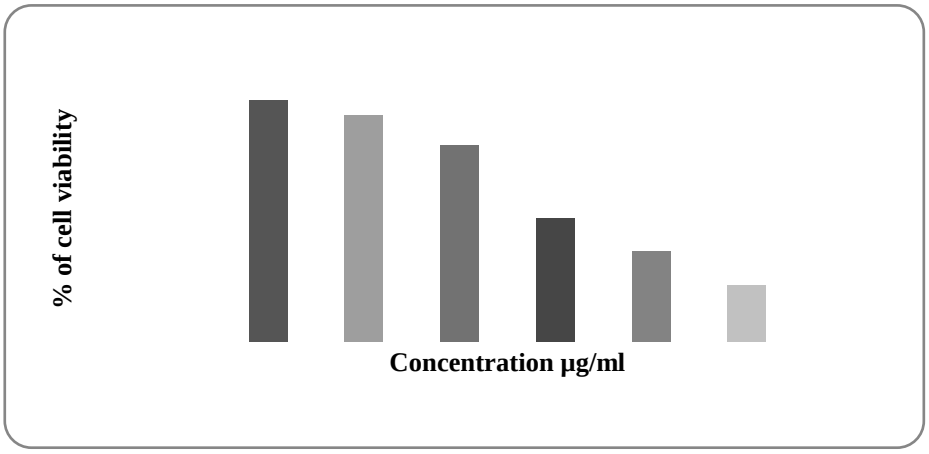

Fig.16 Anticancer Activity of Methanolic Extract

4. CONCLUSION:

In the present study the phytochemical analysis revealed the major compounds which could be explored in future. The DPPH assay exhibited higher antioxidant potential in the methanolic extract compared to hexane. Methanolic extract of L. *inermis* seed showed significant antibacterial activity. The anticancer activity of methanolic extract L. *inermis* seed on MCF 7 cell line showed promising results. The results obtained from this study will be supportive for further research in the field of ethno medicine.

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