Phytochemical screening, Antibacterial and Antioxidant activity of *Melia azedarach*

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ABSTRACT: *Melia azedarach (Kattu vembhu - Tamil) also called as Persian lilac.In the present study, the phytochemical analysis showed that theMelia azedarach contains Alkaloids, Carbohydrate, Tannins, Saponin, Terpenoids, Oxalate and Glycoside and the absent of Amino Acid, Phenol, Flavonoids, Quinones and Coumarin in the Acetone extract. The Benzene extracts of Melia azedarach contains Alkaloids, Carbohydrate, Phenol, Saponin, Terpenoids, Coumarin and Glycoside and absence of Amino Acid, Flavonoids, Tannins, Oxalate and Quinones.Alkaloids, Carbohydrate, Tannins, Saponins, Terpenoids and Oxalate are presentand Amino Acid, Phenol, Flavonoids, Quinones, Coumarin, Glycosides are absent in the Methanol extract.Alkaloids, Amino Acid, Phenol, Flavonoids, Terpenoids, Quinones are presentand Carbohydrates, Tannins, Saponin, Oxalate, Coumarin and Glycosides are absent in the Water extract of Melia azedarach. Anthocyanin is absent in all the solvent extracts of Melia azedarach.Acetone, Benzene, Methanol and Water extract of Melia azedarach show activity against the tested pathogens by the highest zone of inhibition Staphylococcus aureus (10 mm), Escherichia coli (9 mm), Pseudomonas aeruginosa (7 mm) and Bacillus subtilis (5 mm)for Acetone Extract of Melia azedarach, Staphylococcus aureus (6 mm), Bacillus subtilis (5 mm), Escherichia coli (5 mm) and Pseudomonas aeruginosa (4 mm)recorded. Benzene Extract of Melia azedarach resulted inStaphylococcus aureus (16 mm), Escherichia coli (13 mm), Pseudomonas aeruginosa (9 mm) and Bacillus subtilis (6 mm) and for Methanol Extract of Melia azedarach and Pseudomonas aeruginosa (6 mm) and other pathogens are resistant to the Water extract of Melia azedarach.The antioxidant activity level of Melia azedarach solvent extracts was high to Benzene followed by Acetone, Methanol and Water. The results of the study indicate that the Melia azedarach possesses phyto-constituents having antibacterial activity thus it can be utilized as a natural plant based antimicrobials.*

Key Words: *Melia azedarach, phytochemical analysis, Acetone, Benzene, Staphylococcus aureus, Pseudomonas aeruginosa.*

1. INTRODUCTION

Indian greeneries are the chief and cheap sources of medicinal plants and their products. From Centuries till date these medicinal plants have been extensively utilized in AYUSH (Ayurveda Yoga UnaniSiddha Homeopathy). The WHO says about 70% of the population in the developing countries use traditional medicine for the treatment of various ailments(Bharat Pokhrel et al., 2015).It is a species of fast growing and deciduous tree in the mahogany family. The tree is commonly found in central and southern china, India, Sri-Lanka, Nepal, Bhutan, Thailand, Laos, Vietnam, Indonesia, Philippines and Eastern Australia. In South Asia especially in Tamilnadu which known as Malai vembu it has many medicinal uses in the ancient medical practices like Siddha and Ayurveda.The adult tree was commonly measured up to a height of 7–12 Meters, however in exceptional circumstances *M. azedarach* can attain a height of 45 Meters. The branches are grown widely up to 20 Meters. It is alternate, long-petioled, two or three times compound (odd-pinnate) and the leaflets are dark green in the above and lighter green in the below with serrate margins. The flowers are small and fragrant with five pale purple or lilac petals and growing in clusters.The fruit is a drupe, marble-sized, light yellow at maturity and it is highly toxic to warm blooded animals especially ripe fruits are more toxic than green one. All the parts of plant also contain toxin that can cause gastric tract irritation and degeneration of liver and kidney. The roots are superficial and the tree is liable to be blown down under strong winds.Leaf of the plant gives bitter flavour and they are used as the pot-herb in curries and soups. Fruit is edible which gives sweetish flavour and it is eaten by children.The cut branches with mature fruits are sold commercially decoration purposes.An aqueous extract reduces the intensity of asthmatic attacks and the leaf juice is antihelminthic, antilithic and diuretic. The leaves are used externally to treat scabies and itchiness. A decoction is astringent and stomachic, used to treat diarrhea and used as a gargle to treat tooth problems and strengthen the gums. The juice extracted from the leaf is used as a diuretic and to dissolve kidney stones. The juice from leaves is beneficial to cure dandruff. The alkaloids and

anti-inflammatory properties of leaves are well known. It reduces inflammation and pain in the arthritic joint. The decoction prepared from leaves is beneficial to regularize menstrual cycle. The stem bark is antihelminthic, astringent and used as a bitter tonic in India. The fruit is harvested in autumn when it is fully ripe and can be used fresh for medicinal purposes. The aim of the present research work is to evaluate the biological activities of organic and aqueous extracts of *Melia azedarach* species against bacteria, so to evaluate the phytochemical contents and establish the antioxidant potentials.

2.1 COLLECTION OF PLANTS

The leaves of *Melia azedarch* were collected from Thimampettai village, Vaniyambadi, in October 2018. The leaves were identified and authenticated by Dr. N.P.M Mohamed Tariq, Assistant professor of Biotechnology, Islamiah College (Autonomous), Vaniyambadi (**Fig.** 1). After identification, the plant material was processed for extraction.

Fig.1*Melia azedarach*

2.2 PREPARATION OF PLANT EXTRACT

The leaves of *Melia azedarch* were thoroughly cleaned with water to remove dust particles and shade – dried at room temperature and reduced to coarse powder using a mechanical mixer. The powder was subjected to extraction by maceration using various solvents like Acetone, Benzene, Methanol and Water to obtain their respective extracts. To 10gm of the dried plant powder in 100ml solvent (Acetone, Benzene, Methanol, Water) was added and stirred occasionally in orbital shaker. The mixture was filtered on the 2nd day and the solvent was evaporated at room temperature for 18-24 hours to obtain a solid mass, which are stored in refrigerator (4°C) for further use.

2.3. PHYTOCHEMICAL SCREENING:

2.3.1 Alkaloids:

Wagner's test:

1 ml of extract and 1ml of Wagner's reagent are added. Presence of reddish brown precipitate indicates the presence of Alkaloids.

2.3.2 Amino Acid:

Xanthoprotein test:

1 ml of *M. azedarach*plant extract and 1 ml of Con. Nitric Acid are added (white precipitate is formed) it is heated for 2-3 minutes and cooled. Then 1 ml of 20% NaOH is added. Appearance of orange colour indicates the presence of Aromatic Amino Acid.

2.3.3 Carbohydrate:

Molish test

2 ml of extract, 2 ml of Molish reagent and 2 ml of Conc.H_2SO_4 are added. Presence of reddish ring indicates the presence of Carbohydrate.

2.3.4 Phenol:

$FeCl_3$ test:

1 ml of the extract and 1 ml of 5% ferric chloride are added. Appearance of dark green colour / reddish brown / blue / violet / purple indicates the presence of Phenol.

Potassium dichromate test:

2 ml of extract and 1 ml of 10% of potassium dichromate are added. Appearance of red colour

indicates presence of Phenol.

2.3.5 Flavonoids:

Alkaline reagent test:

1 ml of the extract and 1 ml of the 10% of sodium hydroxide are added. Presence of yellow fluorescence colour indicates presence of Flavonoids.

Ammonia test:

1ml of extract, 2ml of 10% of ammonia solution and 1ml of concentrated Sulphuric acid are added. The yellow colour indicates the presence of flavonoids.

2.3.6 Tannins:

$FeCl_3$ test:

2 ml of the extract and 2 ml of the 5% ferric chloride are added. Appearance of green colour indicates the presence of Tannins.

2.3.7 Saponin:

Foam test:

2 ml of the extract and 2 ml of the Dis.H_2O are added and shaken vigorously. Formation of stable foam indicates presence of Saponins.

2.3.8 Terpenoids:

Liebermann-Burchard test:

2 ml of the extract, 2 ml of the chloroform and 2 ml of the acetic acid, 1 ml of the conc.H_2SO_4 are added. Appearance of blue green colour/reddish ring indicates the presence of Terpenoids.

2.3.9 Quinones:-

Hydrochloric acid test:-

1 ml of the extract and 1 ml of the conc.HCL are added. Appearance of yellow colour indicates the presence of Quinone's.

2.3.10 Coumarin:

Sodium hydroxide test:

1 ml of the extract and 1 ml of 10% sodium hydroxide are added. Presence of yellow colour indicates Coumarin.

2.3.11 Glycoside:

Keller-Killiani test:

2 ml of the extract, 2 ml of the glacial acetic acid and few drops of the 5% $FeCl_3$ and conc.H_2SO_4 are added. Presence of reddish brown/blue green colour indicates presence of Glycoside's.

Test for glycoside:

2 ml of extract, 3ml of chloroform and 1ml of 10% ammonia solution are added. Appearance of pink colour indicates presence of glycoside.

2.3.12 Oxalate:

Glacial acetic acid test:

3ml of extract and 1ml of glacial acetic acid are added. Appearance of green colour indicates the presence of oxalate.

2.3.13 Anthocyanin:

Sulphuric acid test:

1 ml of the extract and 1 ml of concentrated Sulphuric acid are added. Appearance of yellowish orange colour indicates presence of Anthocyanin.

2.4 Antibacterial Activity:

The Disc Diffusion Method for Antimicrobial Susceptibility test was used to evaluate the presence of Antibacterial Activities of the different solvent extracts of the plants. The Microorganisms used for the testing are collected from Department of Microbiology, Shanmuga Industries of Arts and Science College, Tiruvannamalai and cultured overnight at 37°C in Nutrient broth. The sterile Muller Hinton Agar (Hi-media) plates were prepared. The 100 mg / ml and 200 mg/ ml of the various solvent extracts were prepared by using the respective solvents. Then 0.2 ml of the bacterial suspension was introduced in to the sterile plates and spreading the bacteria using L-Rod to get an even culture all over the plates. The 6mm Discs was prepared from Whatman No.1 filter paper and it also autoclaved. A plate comprises of four discs, one is positive control, one is negative control and two for the two different concentrations (100, 200 mg / ml) of the same plant extract. Where Ciprofloxacin is used as positive control and respective solvents in which the sample is dissolved was used as negative control. The disc impregnated with positive control, negative control, two different concentration of the plant extract were prepared and placed on the prepared Muller Hinton Agar plates. Then all the plates were kept for incubation at 37°C for 12-18 hours.

2.5. Antioxidant Activity:

The Antioxidant activity of the solvents extracts was performed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. A solution of 0.135mM DPPH was freshly prepared by dissolving 4 mg of DPPH in 100 ml of Methanol. The 2 ml of the different concentrations of extracts (20 µg, 40 µg, 60 µg, 80 µg, 100 µg, 150 µg, 200 µg, 250 µg /ml of respective solvents) is taken and 2 ml of the DPPH solution (0.135mM) was added. The control sample was prepared by 2 ml of DPPH solution alone without sample mixed with 2 ml of methanol. Then all the test tubes were incubated in dark room for 30 minutes at room temperature. The changes in the absorbance (OD values) was measured at 517 nm in UV-Spectrophotometer (Bharat Pokhrel et al., 2015). Methanol only used as blank. The antioxidant activity was expressed in inhibition percentage by using following formula,

$$\text{Inhibition percentage} = \frac{\text{[Control O.D – Sample O.D]}}{\text{Control O.D}} \text{ X 100}$$

3. RESULT AND DISCUSSION

3.1 Phytochemical Screening

The phytochemical analysis of the various solvents extract is tested for 13 phytochemicals**.** The procedure for the phytochemical analysis is carried out using standardized protocols. Where the Acetone extract of the *Melia azedarach* contains Alkaloids, Carbohydrate, Tannins, Saponin, Terpenoids, Oxalate and Glycoside. The Amino Acid, Phenol, Flavonoids, Quinones and Coumarin are absent in the Acetone extract of *Melia azedarach*. Where the Benzene extracts of *Melia azedarach* contains Alkaloids, Carbohydrate, Phenol, Saponin, Terpenoids, Coumarin and Glycoside. Amino Acid, Flavonoids, Tannins,Oxalate and Quinones are absent in Benzene extract. The phytochemicals Alkaloids, Carbohydrate, Tannins, Saponins, Terpenoids and Oxalate are present. Amino Acid, Phenol, Flavonoids, Quinones, Coumarin and Glycosides are absent in Methanol extract of *Melia azedarach*. Alkaloids, Amino Acid, Phenol, Flavonoids, Terpenoids and Quinones are present in Water extract of *Melia azedarach*. Carbohydrates, Tannins, Saponin, Oxalate, Coumarin and Glycosides are absent in Water extract of *Melia azedarach*. Anthocyanin is absent in all the solvent extracts of *Melia azedarach***(Table 1)** as observed by Abdul Viqar Khan et al., (2011) and Melkamu Feyera Fufa et al., (2018).

3.2 Antibacterial Activity

The Disc Diffusion Method for Antimicrobial Susceptibility test was used to evaluate the presence of Antibacterial Activities of the different solvent extracts of *Melia azedarach*. The *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus* are used for testing. Bacteria are cultured overnight at 37°C in Nutrient broth. Sterile Muller Hinton Agar (Hi-media) plates were prepared. The 150 mg / ml and 300 mg/ ml of the Acetone, Benzene, Methanol, Water solvent extracts were prepared by using the respective solvents. Then 200µl of the bacterial suspension was introduced into the sterile plates and spreading the bacteria using L-Rod to get an even culture all over the plates. The 6mm Discs was prepared from Whatman No.1 filter paper and it also autoclaved. A plate comprises of four discs, one is positive control, one is negative control and two for the two different concentrations (150mg/ml, 300 mg / ml) of the same plant extract. The sterile discs are placed above the media and the samples are loaded gently by the following quantity given below. Where 10µl of Ciprofloxacin (1mg/ml) is loaded as positive control in the disc and 10µl of the respective solvents in which the sample is dissolved was used as negative control in the disc. Then 20µl of the 150 mg/ ml and 300 mg/ ml prepared concentration of the extract is loaded gently in the following discs. Then all the plates are kept for incubation at 37°C for 24 hours**.** Acetone, Benzene, Methanol and Water extract of *Melia azedarach* showed activity against the tested pathogens by the following order from the highest zone of inhibition *Staphylococcus aureus* (10 mm), *Escherichia coli* (9 mm), *Pseudomonas aeruginosa* (7 mm) and *Bacillus subtilis* (5 mm)for Acetone Extract of *Melia azedarach, Staphylococcus aureus* (6 mm), *Bacillus subtilis* (5 mm), *Escherichia coli* (5 mm) and *Pseudomonas aeruginosa* (4 mm)for Benzene Extract of *Melia azedarach*, *Staphylococcus aureus* (16 mm), *Escherichia coli* (13 mm), *Pseudomonas aeruginosa* (9 mm) and *Bacillus subtilis* (6 mm) for Methanol Extract of *Melia azedarach* and *Pseudomonas aeruginosa* (6 mm) and other pathogens are resistant to the Water extract of *Melia azedarach***(Table 2,Fig. 2 & 3)** as observed Abdul Viqar Khan et al., (2011), Anterasen and Amla Batra (2012), Bharat Pokhrel et al., (2015) and Melkamu Feyera Fufa et al., (2018).

3.3 Antioxidant activity

The antioxidant activity of the *Melia azedarach* Acetone, Benzene, Methanol and Water solvent extracts is tested by using 2,2-diphenyl-1-picrylhydrazyl radical(DPPH) method and it is compared with

standard Ascorbic Acid. Antioxidant activity is very important in counteracting the deleterious free radical in food biological system. The DPPH alcohol solution is a deep purple colour with an absorption peak at 517 nm which disappears in the presence of radical scavengers in the reactive system. The scavenging capacity of biological reagents on DPPH free radical can be expressed as its antioxidant capability. Evidences collected in recent year suggest the involvement of free radicals and other oxidants as the major cause of oxidative stress that leads to a variety of diseases and disorders. This led to an increasing interest in natural products having antioxidant properties. Plants have been considered as richer sources of antioxidants. In our study the antioxidant activity of the *Melia azedarach* solvents extracts was high to Benzene followed by Acetone, Methanol and Water as observed by Bharat Pokhrel et al., (2015) and Mahmood Ahmad et al., (2017) and the O.D value (**Table 3**) of the tested samples was used to calculate the percentage of Inhibition and Standard Error**.**

4. Summary and Conclusion

*M. azedarach*is a large evergreen tree native to India, growing wild in the sub-Himalayan region. The United Nations declared Neem as the "Tree of the 21st Century" as it consists of high potential medicinal values. It has long been using in pharmacy, health, beauty, pet care, pesticides and insecticides, and agriculture. *M. azedarach* was found to be rich in alkaloids and also with phenolic compounds which shows there will be a high content of medicinal important bioactive compounds. Various extracts shows the presence of high concentration of terpenoids compounds which are the main sources of steroids. DPPH antioxidant activity of the various extracts exhibited good results where it shows the presence of rich antioxidants which can serve as a good source for immunity development.

Table 1 Phytochemical Screening of *Melia azedarach*.

S.No	Phytochemical's	Test Names	Acetone	Benzene	Methanol	Water
1	**Alkaloids**	Wagner's test	+	+	+	+
2	**Amino Acid**	Xanthoprotein test	-	-	-	+
3	**carbohydrate**	Molish's test	+	+	+	-
4	**Phenol**	a)$FeCl_3$ test	-	+	-	+
		b)Potassium dichromate	-	+	-	+
5	**Flavanoids**	Alkaline reagent test	-	-	-	+
		Ammonia test	-	-	-	+
6	**Tannins**	$FeCl_3$ test	+	-	+	-
7	**Saponin**	Foam test	+	+	+	-
8	**Terpenoids**	a)Salkowskis test	+	+	+	+
		b)Liberman burchard's test	+	+	+	+
9	**Oxalate**	Glacial acetic acid test	+	-	+	-

10	**Quinones**	Hydrochloric acid test	**-**	**-**	**-**	**+**
11	**Coumarin**	Sodium hydroxide test	**-**	**+**	**-**	**-**
12	**Glycoside**	Kellar Killanis test	**+**	**+**	**-**	**-**
13	**Anthocyanins**	Sulphuric acid test	**-**	**-**	**-**	**-**

Presence (+), Absence (-)

Table 2 Antibacterial activity of *Melia azedarach*

Name of the Microorganism	Solvents	Zone of Inhibition in mm			
		*P.C	*N.C	100 mg/ml	200 mg/ml
Bacillus subtilis	Acetone	13mm	-	5mm	8mm
	Benzene	17mm	-	6mm	8mm
	Methanol	17mm	-	6mm	8mm
	Water	7mm	-	-	-
Escherichia coli	Acetone	14mm	-	9mm	11mm
	Benzene	17mm	-	5mm	6mm
	Methanol	14mm	-	13mm	15mm
	Water	14mm	-	-	-
Pseudomonas aeruginosa	Acetone	13mm	-	7mm	9mm
	Benzene	13mm	-	4mm	7mm
	Methanol	13mm	-	9mm	15mm
	Water	13mm	-	6mm	6mm
Staphylococcus aureus	Acetone	12mm	-	10mm	11mm
	Benzene	12mm	-	6mm	8mm
	Methanol	11mm	-	16mm	18mm
	Water	16mm	-	-	-

PC=Positive control (Ciproflaxin) **NC**=Negative control (Respective solvents)

Table 3 Antioxidant value of *Melia azedarach* extracts at 517nm.

Concentration	Ascorbic Acid	Acetone	Benzene	Methanol	Water
20 µg / ml	0.0159	0.5296	0.5009	0.5329	0.5329
40 µg / ml	0.0143	0.4945	0.4878	0.5109	0.4519
60 µg / ml	0.0125	0.4517	0.4490	0.4918	0.4348
80 µg / ml	0.0108	0.4199	0.3764	0.4755	0.4205
100 µg / ml	0.0077	0.3731	0.3535	0.4465	0.4069
150 µg / ml	0.0070	0.2234	0.2052	0.2674	0.4004
200 µg / ml	0.0059	0.1833	0.1480	0.2241	0.3925
250 µg / ml	0.0049	0.1283	0.1088	0.1749	0.3660

A high antibacterial activity was found in methanol and acetone extracts of *M. azedarach* compared to others against bacterial pathogens like *E. coli, B. subtilis, P. aeruginosa* and *S. aureus.* With this studies it is obvious that the species *M. azedarach* found to be a great source ofvarious medicinally important bioactive compounds.In addition has good nutritional, antioxidant and antibacterial activities.

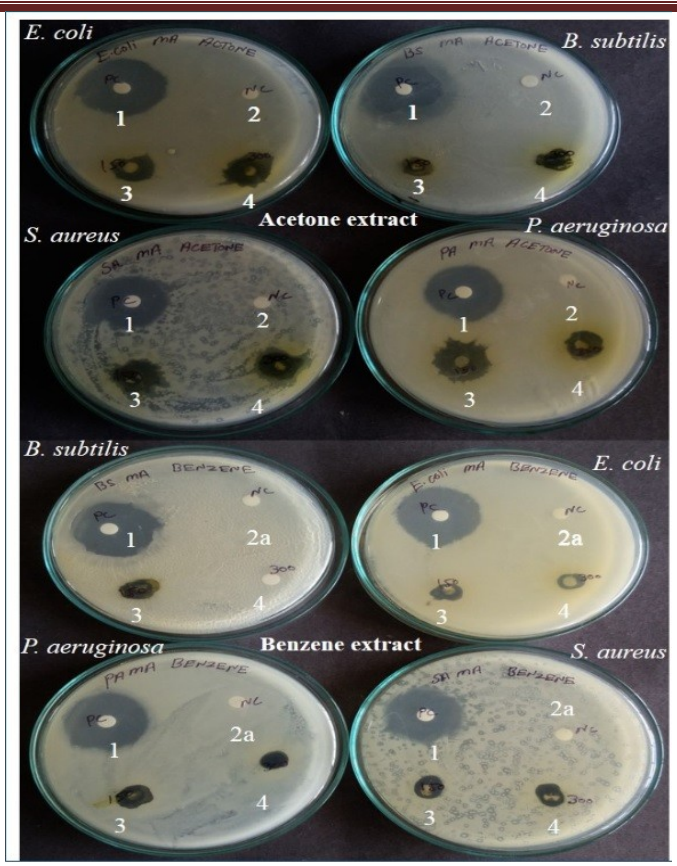

Fig.2. Antibacterial activity of *Melia azedarach* extracts. 1 – Positive Control (Ciprofloxacin 1mg/ml),2-Negative Control (Acetone),2a - Negative Control (Benzene),3–150mg/mlof *Melia azedarach*extract & 4-300mg/ml of *Melia azedarach* extract.

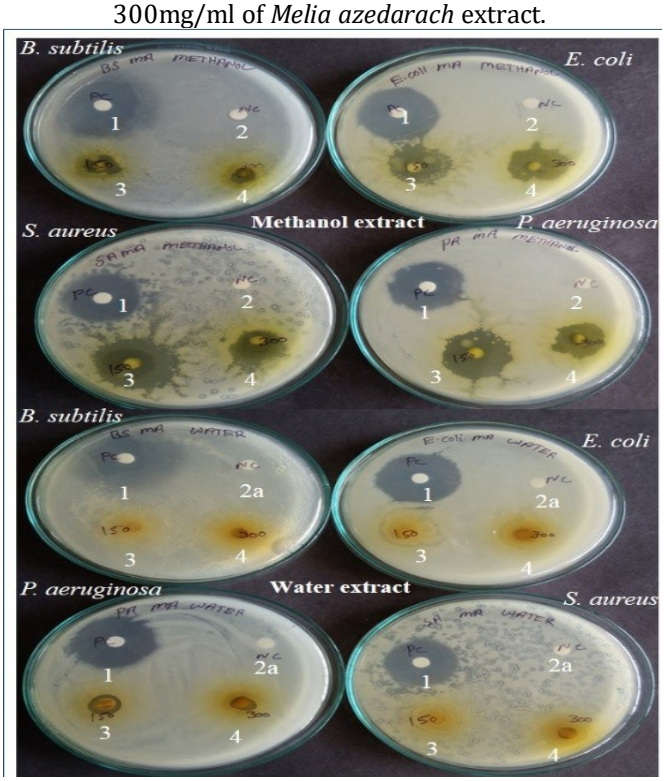

Fig.3. Antibacterial activity of *Melia azedarach* extracts. 1 – Positive Control (Ciprofloxacin 1mg/ml), 2-Negative Control (Methanol), 2a - Negative Control (Water), 3 –150mg/ml of *Melia azedarach* extract & 4-300mg/ml of *Melia azedarach* extract.

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