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Research Paper

# Evaluation of antimicrobial activity and preliminary phytochemical screening of M*esua ferrea* seeds extract

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## ABSTRACT
The present study was conducted to evaluate the antimicrobial activity and phytoconstituents of hexane, methanol and aqueous extracts of seeds of *Mesua ferrea* Linn. In vitro. The antimicrobial activity of different extracts was tested against the gram positive and the gram negative bacterial strains and some fungal strains by observing the zone of inhibition diameter. The gram positive bacteria used in the test were *Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Bacillus cereus* and the gram negative bacteria were *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris*, fungal strains like *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* were used. *Proteus vulgaris* and *Bacillus subtilis* were the most sensitive strain to the hexane and methanolic extract of *Mesua ferrea* and exhibited the maximum zone of inhibition diameter. *Mesua ferrea* did not show any activity against *Escherichia coli*. methanolic extract of *Mesua ferrea* was most sensitive against *Aspergillus niger* and *Aspergillus flavus* with maximum inhibitory zone diameter exhibited against *Aspergillus niger* and *Aspergillus flavus*. *Mesua ferrea* seeds extracts were inactive against *Candida albicans.* Phytochemical screening showed the presence of alkaloids, saponins, phenolics, tannins, flavonoids and terpenoids. The results confirmed the antimicrobial activity of *Mesua ferrea* seeds extracts against various human pathogenic bacteria and fungus.

**Keywords**: Phytochemicals; Crude extract; Antimicrobial activity; *Mesua ferrea* Linn.

## INTRODUCTION
In recent years, numerous drug resistances in human pathogenic microorganisms have developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Khan, et al., 2003). Ethno pharmacologists, botanists, microbiologists, and natural-product chemists are searching the earth for phytochemicals which could be developed for the treatment of infectious diseases especially in light of the emergence of drug-resistant microorganisms and the need to produce more effective antimicrobial agents (Tanaka, et al., 2006) The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources. Different extracts from traditional medicinal plants have been tested to identify the

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sources of the therapeutic effect. As a result some natural products have been approved as new antimicrobial drugs.

Researchers have shown that all different parts of the plants which include; stem, root, leaves, flowers, seeds etc possess antimicrobial property (Bibi, et al., 2005). *M. ferrea* is well documented for its biological activities such as Cytotoxic activity of *Mesua ferrea* has been reported against human cholangiocarcinoma, laryngeal and hepatocarcinoma cells in vitro (Wanna, et al., 2010), *In-vivo* antioxidant activity and hepatoprotective effects of *M. ferrea* has been reported in ethanolic extract (Sandeep, et al., 2009), Anti-inflammatory and C.N.S. depressant activities of xanthones from *Mesua ferrea* was reported (Gopalakrishnan, *et al.,* 1980), pet ether extracts of leaf of M. nagassarium also shows highly antibacterial activity (Shikder, et al., 2011),antibacterial potential of Mesua ferrea Linn. flowers (Mazumder, et al. 2004), Supercritical $CO_2$ selectively extracted a series of 4-alkyl and 4-phenyl 5,7-dihydroxycoumarins from *Mesua ferrea* blossoms, the isolated compounds showed weak antiprotozoal and potent antibacterial activity (Luisella *et al.,* 2004).

The plant *Mesua ferrea* Linn. (*Guttifereae*). also known as Parag nagkesar, Cobra saffron. is found in moist, evergreen or semi-evergreen forests, It is native to tropical Sri Lanka but also cultivated in Assam, southern Nepal, Indochina, India and the Malaya Peninsula. *Mesua* seeds contain pale yellow lactone, mesuol, phenolic constituent, mesuone in small amounts, seed kernel is rich in phosphorous and nitrogen. The seed oil is considered to be very useful in conditions like vata and skin diseases. Dried flowers are used for bleeding haemorrhoids and dysentery with mucus. Fresh flowers are useful remedy for itching, nausea, bleeding piles, metrorrhagea, menorrhoea, excessive thirst, and sweating, for sores, scabies, wounds, and rheumatism, dried flowers also used for preparation of perfumed ointments. The flowers and leaves are used in Bengal as an antidote to snake poison. Its dried flowers are ground to form a powder and after mixing with ghee or their paste with butter and sugar are given in bleeding piles and dysentery in different parts of India (Jadhav Dinesh, 2008; Wealth of India, 1992).

The aim of present work was to compare the efficacy of *M. ferrea* against the tested bacterial and fungal strains using the agar well diffusion method for determination of Zone of inhibition and minimal inhibitory concentration (MIC) method. The *M. ferrea* seeds could be of great importance as it can be used as an effective alternate antibacterial against multi-drug resistant pathogens.

## MATERIALS AND METHODS

***Plant Material*****:** The seeds of *Mesua ferrea* plant were collected from the botanical garden of Forest Research Institute, Dehradun (Uttarakhand), India in the month of April 2011. The seeds were authenticated by Mr. S.K Srivastava, Scientist D/HOO, Botanical Survey of India. Dehradun (U.K.), India.

***Extraction procedure*****:** The collected plant material were dried under shade and powdered using an electric blender. The 1g/1ml of the powdered sample was transferred to closed containers. The powdered samples were than extracted by means of cold extraction process using the solvents water, methanol and hexane separately for 72 hours by using magnetic stirrer and also by occasional shaking by hand at room temperature, each of the extracts was filtered by using muslin cloth and then by Whatman no.1 filter paper, the filtrate was evaporated to dryness in an evaporating dish on a steam bath at 50 – $60^o$ C. The extract was then further used in the test.

***Preliminary Phytochemical Screening*****:** The freshly prepared extracts were subjected to standard phytochemical analysis to test for the presence of the phytoconstituents like tannins, saponins, alkaloids, carbohydrates, glycosides, phenolics, flavonoids and terpenoids.

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Chemical tests were carried out on hexane, methanol and aqueous extracts of the powdered specimens using standard procedures for identification of their phytochemical constituents. (Raman, 2006; Evans, 2008)

***Test for Alkaloids:*** Solvent free extract, 20mg was stirred with few ml of dilute hydrochloric acid and filtered the filtrate is tested carefully with alkaloidal reagents as follows:

***Mayer's reagent test*:** Mercuric chloride (1.4g) was dissolved in 60ml of water and potassium iodide (5.0g) was dissolved in 10ml of water, the two solutions were mixed to make volume up to 100ml with water. To a few ml of filtrate, a drop or two of Mayer's reagent was added by the side of the tube. A white or creamy precipitate appears and indicates positive test.

***Dragendorff's reagent test*:** Bismuth carbonate (5.2g) and sodium iodide (4g) are boiled for a few min with 50ml of glacial acetic acid. Filtered, clear red-brown filtrate, 40ml was mixed with 160ml ethyl acetate and 1ml water, 10ml of this solution is mixed with 20ml of acetic acid and made up to 100ml with water. To a few ml of filtrate 10ml of this reagent was added, a prominent yellow precipitate was formed indicating positive test.

***Test for Carbohydrates*:** The extract (50mg) was dissolved in 2.5ml of water and filtered, the filtrate was subjected to the following tests:

***Benedict's test*:** To 0.5ml of filtrate, 0.5ml of benedict's reagent (Sodium citrate-173g and sodium carbonate-100g dissolved in 800ml of distil water and boiled to make it clear, copper sulphate-17.3g dissolved in 100ml of distilled water was added to it), boiled for 2min. A brick red coloured precipitate indicates the presence of sugar.

***Molish's test*:** To 2ml of filtrate, two drops of alcoholic solution of α - naphthol are added and shaken well and 1ml of sulphuric acid was added slowly by the side of the test tube. A violet ring indicates the presence of carbohydrates.

***Test for Glycosides*:** 50mg of the extract was hydrolysed with concentrated hydrochloric acid for 2h on a water bath, filtered and filtrate was subjected to following tests:

***Borntragor's test*:** To 2ml of filtrate 3ml of chloroform was added and shaken, chloroform layer was separated and 10% of ammonia solution was added to it. Formation of pink or red colour in ammonical layer indicates the presence of glycosides.

***Legal's tests*:** 50mg of the extract was dissolved in pyridine and sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide was indicated by pink colour.

***Test for saponin*:** About 50g of the extract was diluted in 20ml of distilled water and shaken vigorously in a graduated cylinder for 15min. A 2cm layer of foam indicates the presence of saponins.

***Test for phenolics and tannins*:**

***Ferric chloride test*:** The 50mg is dissolved in 2 ml of distilled water. A few drops of 0.1% ferric chloride were added. A dark green colour indicates the presence of phenolics compounds.

***Lead acetate test*:** The 50mg of extract was dissolved in distilled water and 3% of lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

***Test for flavonoids*:** 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated $H_2SO_4$. A yellow colouration indicated the presence of flavonoids. The yellow colouration disappeared on standing.

***Test for terpenoids*:** Five ml of each extract was mixed in 2ml of chloroform, and concentrated $H_2SO_4$ (3ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

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***Microorganisms used*:** The microorganisms include gram negative bacteria *Escherichia coli* (MTCC 452), *Pseudomonas aeruginosa* (MTCC 1034), *Proteus vulgaris* (MTCC 1771), gram positive bacteria *Staphylococcus aureus* (MTCC 737), *Bacillus subtilis* (MTCC 441), *Enterococcus faecalis* (MTCC 439), *Bacillus cereus* (MTCC 1306) and fungal strains *Aspergillus niger* (MTCC 1344), *Aspergillus flavus* (MTCC 277), *Candida albicans* (MTCC 227), were obtained from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India.

***Preparation of extracts*:** The crude extracts were dissolved in 30% dimethyl sulphoxide (DMSO) and further diluted to obtain of each extracted sample of 100mg/L concentration was used for the determination of antibacterial and antifungal activity.

***Preparation of media and inoculums*:** Nutrient Agar/broth and Potato Dextrose Agar/broth (Himedia, India) were used as the media for culturing of bacterial and fungal strains. A loopful of bacterial pure culture was inoculated in 20ml sterile nutrient broth medium in the test tubes aseptically and this process was repeated for all the bacterial strains. The tubes were incubated at $37^0$ C for 24 hr. growth was observed in all the test tubes and this was further used in the experiment.

***Standard antibiotic*:** Ciprofloxacin 0.3% w/v.

***Standard antifungal*:** Itraconazole (25mcg), Clotrimazole (25mcg).

***Antibacterial activity: Agar well diffusion method*:** About 1ml of the inoculum was poured in the sterilized nutrient agar media when media attains a temperature of 30-40$^o$C, mixed well, and 20ml of this media was poured in all the petriplates and allowed to solidify. Then four wells of 6mm were made in each petriplate with the help of a sterile cork borer, 50µl of the plant extract was poured in each well using sterilized micropipettes. For each bacterial strain, negative controls were maintained where pure solvents were used instead of the extract. The control zones were subtracted from the test zones, for positive control, standard antibiotic Ciprofloxacin (30- mcg) was used. The plates were incubated overnight at 37°C. Microbial growth was determined by measuring the diameter of zone of inhibition. The entire process was carried out aseptically in the laminar airflow. The experiment was performed in triplicate (Subban, et al., 2011)

***Antifungal activity: Agar well diffusion method*:** About 1ml of the inoculum was poured in the sterilized Potato dextrose agar media when media attains a temperature of 30-40$^o$C, mixed well, and 20ml of this media was poured in all the petriplates and allowed to solidify. Then four wells of 6mm were made in each petriplate with the help of a sterile cork borer, 50µl of the plant extract was poured in each well using sterilized micropipettes. For each fungal strain, negative controls were maintained where pure solvents were used instead of the extract. The control zones were subtracted from the test zones, for positive control, standard antifungal Itraconazole (25-mcg) and Clotrimazole (25-mcg) were used. The plates were incubated 28°C for 48–72hrs. Fungal growth was determined by measuring the diameter of the zone of inhibition. The entire process was carried out aseptically in the laminar airflow. The experiment was performed in triplicate (Varaprasad, et al., 2009)

***Determination of Minimum Inhibitory Concentration (MIC)***

MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation.

MIC by “Serial Tube Dilution Method” In this technique the tubes of broth medium, containing graded doses of compounds are inoculated with the test organism. After suitable incubation, growth will occur in those tubes where the concentration of compound is below the inhibitory level and the culture will become turbid (cloudy). Therefore, growth will not occur above the inhibitory level and the tubes will remain clear.

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***Preparation of the sample solution*:** 0.64g of the solvent free extract was weighed and dissolved in 100ml of solvent (DMSO) having concentration 640mcg/ml and further dilutions were made from this sample and likewise.

***Preparation of inoculums*:** The test bacteria grown at $37^o$ C in nutrient agar medium was diluted in sterile nutrient broth by taking one loopful of test bacterial strain in 20ml of nutrient broth, and the fungal strain grown in potatoes dextrose agar at $28^o$ C for 72hrs. was diluted in sterile potatoes dextrose broth . This suspension was used as inoculum.

***Procedure:*** Nine test tubes were taken, and marked 1,2,3,4,5,6,7 and the rest two were assigned as TMC (Medium + Compound) and TMI (medium + Inoculum). 100ml of nutrient broth(for bacteria) and 100ml of potatoes dextrose broth (for fungus) was made and sterilized in an autoclave for 15Ibs/sq. inch pressure for 15min. 9ml of sterilized nutrient broth was poured in each of the 9 test tubes.

1ml of the sample solution was added to the 1st test tube and mixed well and then 1ml of this content was transferred to the 2nd test tube. The content of the second test tube was mixed well and again 1ml of this mixture was transferred to the 3rd test tube. This process of serial dilution was continued up to the 7 test tubes and mixed well. 10µl of the properly diluted inoculum was added to each of 7 test tubes and mixed well. To the control test tube TMC, 1ml of the sample was added and mixed well and 1ml of this mixed content was discarded to check the clarity of the medium in presence of diluted solution of the compound. 10µl of the inoculum was added to the control test tube TMI, and observed the growth of the organism in the medium used. All the test tubes were incubated at $37^o$ C for 18-24hrs for bacterial growth and at $28^o$ C for 72h. for fungal growth. The entire experimental process was carried out aseptically in the laminar flow (Gislene, et al., 2000; Jenifer, 2001)

## RESULTS AND DISCUSSION

Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent. Phytochemical investigations of *M. ferrea* hexane, methanol and aqueous extracts with various specific reagents showed the presence of alkaloids, saponins, phenolics, tannins, flavonoids and terpenoids. Methanolic extract of *Mesua ferrea* contains maximum number of phytoconstituents including of alkaloids, glycosides, saponins, phenolics, tannins, flavonoids and terpenoids. The hexane extract contains flavonoids and terpenoids whereas, its aqueous extract contains only saponins. The extract showed concentration dependent antibacterial activity against gram positive and gram negative bacterial strains. The hexane and methanol extract showed maximum sensitivity towards different bacterial strains whereas the aqueous extract showed least sensitivity, *P. vulgaris* and *B. subtilis* were the most sensitive strain to the hexane extract with zone of inhibition diameter of 22mm and 24mm and to the methanolic extract with zone of inhibition diameter of 24mm and 23mm. According to Parekh and Chanda 2008, antimicrobial activity of methanol extract of mesua seeds was 12mm and 13 mm against *A. nige*r and *A. flavus* at 125µg and 250µg which is comparatively very low to the present results obtained at a lower concentration of 50µg.

It was observed that *M. ferrea* did not show any activity against *E. coli*. Aqueous extract demonstrated activity only against *A. niger* with zone of inhibition diameter 24mm, methanolic extract of seeds exhibited remarked antifungal activity against *A. niger* and *A. flavus* with zone of inhibition diameters of 26mm and 22mm whereas, hexane extract of the seeds did not exhibited any antifungal activity against any of the test fungal strains. The standard antibiotic Ciprofloxacin 30µg taken as positive control showed response against all bacterial strains used in the test. The antifungal Clotrimazole demonstrated a higher activity compared to Itraconazole as it was effective against all the fungal strains tested, while

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Itraconazole was effective only against *Aspergillus flavus* and *Candida albicans*. Concerning the zone of inhibition, the values were merely higher for those caused by antibiotics respect to those caused by the plant extracts, the minimum inhibitory concentration (MIC) required to inhibit the growth of organisms were measured in this study. The MIC values of *M. ferrea* hexane extract against *S. aureus, Proteus vulgaris, B.subtilis*, *E. faecalis*, *B, cereus* are 40, 20, 80, 20, 40 µg/ml. MIC values of *M. ferrea* methanol extract against *S. aureus, P. aeruginosa*, *Proteus vulgaris, B. subtilis*, *E. faecalis*, are 40, 80, 40, 80, 40 µg/ml. The MIC values of *M. ferrea* methanol extract against *A. niger* is 40µg/ml and *A. flavus* is 40µg/ml. The minimum inhibitory concentration of the leaf extract was determined by visual observations and it was observed that the MIC for the bacterial strains was in the range of 20µg to 80µg.

Antibacterial activities of aqueous, ethanol and hexane extract of *M. Ferrea* seeds were compared in this study (Table 2, 5). The zones of inhibition of growth of the microorganisms are a function of relative antibacterial activity of the extracts. The extracts showed selective levels of activities against the organisms. The methanolic and hexane extract of the seeds considered in the study possess maximum phyto constituents as well as maximum number of compounds. Strong antimicrobial activity is seen in methanolic extract as compared to the hexane extract and least in the aqueous extract which draws a conclusion that maximum number of phyto constituents responsible for the activity are extracted out in hexane and methanol extract due to the volatile or essential oil containing nature of *Mesua ferrea* seeds. Aqueous extract showed minimal antibacterial activity and the variation in activities of the extracts may be due to insolubility of active compounds in water as compared to methanol and hexane.

No microbial growth was observed in the test tubes TM (containing medium only) and TMC (medium + test sample, no inoculum) indicating that the medium and the test sample were not contaminated by microorganism and the total investigation was performed properly in the sterile condition. Microbial growth was observed in the test tube TMI (medium + inoculums) revealing the fact that there was no problem with the sub-cultured microorganisms. The broad spectrum antimicrobial action displayed by some of these extracts could be attributed to the presence of pronounced antimicrobial phyto constituents such as terpenoids, tannins, flavonoids, alkaloids and phenols (Table 1).The results obtained in the present study supports the plants contain active biological compounds with effective in resisting the growth of bacteria.

## CONCLUSION

The extract obtained with methanol and hexane was found to have a better effectiveness against the tested bacteria and fungi. Our data express that the *M. ferrea* seeds have great potential as an antimicrobial against the microorganisms tested. Thus, it can be used in the treatment of infectious diseases caused by resistant microbes.

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**Table- 1: Phytochemical screening of *Mesua ferrea* seeds extracts.**

| Tests | Hexane extract | Methanol extract | Aqueous extract |
|---|---|---|---|
| Alkaloids | | | |
| Dragendorff's test | _ | + | _ |
| Mayer's test | _ | + | _ |
| Carbohydrates | | | |
| Molisch's test | _ | _ | _ |
| Benedict's test | _ | _ | _ |
| Glycosides | | | |
| Borntrager's test | _ | + | _ |
| Legal's test | _ | + | _ |
| Saponins | _ | + | + |
| Phenolics& Tannins | | | |
| Ferric chloride test | _ | + | _ |
| Lead acetate test | _ | + | _ |
| Flavonoids | + | + | _ |
| Triterpenoids | + | + | _ |

- (+) sign indicates the presence of chemical constituents; ( - ) sign indicates the absence of chemical constituents.

**Table- 2**: **Determination of antibacterial activity** (including bore diameter 6mm).

| Bacterial Strains | Zone of Inhibition (in mm) | | | Antibiotic Cp (30 mcg) |
|---|---|---|---|---|
| | Hexane | Methanol | Aqueous | |
| ***Staphylococcus aureus*** | 20 | 18 | 16 | 28 |
| ***Escherichia coli*** | _ | _ | _ | 28 |
| ***Pseudomonas aeruginosa*** | _ | 17 | _ | 26 |
| ***Proteus vulgaris*** | 22 | 24 | _ | 20 |
| ***Bacillus subtilis*** | 24 | 23 | _ | 18 |
| ***Enterococcus faecalis*** | 19 | 22 | _ | 21 |
| ***Bacillus cereus*** | 20 | _ | _ | 21 |

- Cp – Ciprofloxacin ; (-) no activity.

**Table- 3A: MIC of *Mesua ferrea* hexane extract.**

| Marked no. of Test tubes | Nutrient broth added (ml) | *Mesua* Hexane extract (μg/ml) | Inoculums added (μl) | Bacterial growth | | | |
|---|---|---|---|---|---|---|---|
| | | | | *S. aureus* | *E. coli* | *P. aeruginosa* | *P. vulgaris* |
| 1 | 1 | 640 | 10 | _ | _ | _ | _ |
| 2 | 1 | 320 | 10 | _ | _ | _ | _ |
| 3 | 1 | 160 | 10 | _ | _ | _ | _ |
| 4 | 1 | 80 | 10 | _ | _ | _ | _ |
| 5 | 1 | 40 | 10 | _ | _ | _ | _ |
| 6 | 1 | 20 | 10 | + | _ | _ | _ |
| 7 | 1 | 10 | 10 | + | _ | _ | + |
| $T_{MC}$ | 1 | 640 | 0 | _ | _ | _ | _ |
| $T_{MI}$ | 1 | 0 | 10 | + | + | + | + |

- (+) Turbidity present ; (-)Turbidity absent.

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**Table- 3B: MIC of *Mesua ferrea* hexane extract.**

| Marked no. of Test tubes | Nutrient broth added (ml) | *Mesua* Hexane extract (µg/ml) | Inoculums added (µl) | Bacterial growth | | |
|---|---|---|---|---|---|---|
| | | | | *B. Subtilis* | *E. faecalis* | *B. cereus* |
| 1 | 1 | 640 | 10 | _ | _ | _ |
| 2 | 1 | 320 | 10 | _ | _ | _ |
| 3 | 1 | 160 | 10 | _ | _ | _ |
| 4 | 1 | 80 | 10 | _ | _ | _ |
| 5 | 1 | 40 | 10 | + | _ | _ |
| 6 | 1 | 20 | 10 | + | _ | + |
| 7 | 1 | 10 | 10 | + | + | + |
| $T_{MC}$ | 1 | 640 | 0 | _ | _ | _ |
| $T_{MI}$ | 1 | 0 | 10 | + | + | + |

**Table- 4A: MIC of *Mesua ferrea* methanol extract.**

| Marked no. of Test tubes | Nutrient broth added (ml) | *Mesua* Methanol extract (µg/ml) | Inoculums added (µl) | Bacterial growth | | | |
|---|---|---|---|---|---|---|---|
| | | | | *S. aureus* | *E. coli* | *P. aeruginosa* | *P. vulgaris* |
| 1 | 1 | 640 | 10 | _ | _ | _ | _ |
| 2 | 1 | 320 | 10 | _ | _ | _ | _ |
| 3 | 1 | 160 | 10 | _ | _ | _ | _ |
| 4 | 1 | 80 | 10 | _ | _ | _ | _ |
| 5 | 1 | 40 | 10 | _ | _ | + | _ |
| 6 | 1 | 20 | 10 | + | _ | + | + |
| 7 | 1 | 10 | 10 | + | _ | + | + |
| $T_{MC}$ | 1 | 640 | 0 | _ | _ | _ | _ |
| $T_{MI}$ | 1 | 0 | 10 | + | + | + | + |

**Table- 4B: MIC of *Mesua ferrea* methanol extract.**

| Marked no. of Test tubes | Nutrient broth added (ml) | *Mesua* Methanol extract (µg/ml) | Inoculums added (µl) | Bacterial growth | | |
|---|---|---|---|---|---|---|
| | | | | *B. Subtilis* | *E. faecalis* | *B. cereus* |
| 1 | 1 | 640 | 10 | _ | _ | _ |
| 2 | 1 | 320 | 10 | _ | _ | _ |
| 3 | 1 | 160 | 10 | _ | _ | _ |
| 4 | 1 | 80 | 10 | _ | _ | _ |
| 5 | 1 | 40 | 10 | + | _ | _ |
| 6 | 1 | 20 | 10 | + | + | _ |
| 7 | 1 | 10 | 10 | + | + | _ |
| $T_{MC}$ | 1 | 640 | 0 | _ | _ | _ |
| $T_{MI}$ | 1 | 0 | 10 | + | + | + |

**Table- 5: Determination of Antifungal activity** (including 6mm bore diameter).

| Fungal Strains | Zone of Inhibition Diameter (mm) | | | Std. Antifungal I 25mcg/ml | Std. Antifungal II 25mcg/ml |
|---|---|---|---|---|---|
| | Hexane | Methanol | Aqueous | | |
| *Aspergillus niger* | _ | 26 | 24 | _ | 25 |
| *Aspergillus flavus* | _ | 22 | _ | 21 | 23 |
| *Candida albicans* | _ | _ | _ | 17 | 27 |

- Standard I : Itraconazole; Standard II : Clotrimazole

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**Table- 6: MIC of *Mesua ferrea* methanol extract.**

| Marked no. of Test tubes | Nutrient broth added (ml) | *Mesua* Methanol extract (µg/ml) | Inoculums added (µl) | Fungal growth | | |
|---|---|---|---|---|---|---|
| | | | | *A. niger* | *A. flavus* | *C. albicans* |
| 1 | 1 | 640 | 10 | _ | _ | _ |
| 2 | 1 | 320 | 10 | _ | _ | _ |
| 3 | 1 | 160 | 10 | _ | _ | _ |
| 4 | 1 | 80 | 10 | _ | _ | _ |
| 5 | 1 | 40 | 10 | + | + | _ |
| 6 | 1 | 20 | 10 | + | + | _ |
| 7 | 1 | 10 | 10 | + | + | _ |
| $T_{MC}$ | 1 | 640 | 0 | _ | _ | _ |
| $T_{MI}$ | 1 | 0 | 10 | + | + | + |

**Table- 7: MIC of *Mesua ferrea* aqueous extract.**

| Marked no. of Test tubes | Nutrient broth added (ml) | *Mesua* aqueous extract (µg/ml) | Inoculums added (µl) | Fungal growth | | |
|---|---|---|---|---|---|---|
| | | | | *A. niger* | *A. flavus* | *C. albicans* |
| 1 | 1 | 640 | 10 | _ | _ | _ |
| 2 | 1 | 320 | 10 | _ | _ | _ |
| 3 | 1 | 160 | 10 | _ | _ | _ |
| 4 | 1 | 80 | 10 | _ | _ | _ |
| 5 | 1 | 40 | 10 | + | _ | _ |
| 6 | 1 | 20 | 10 | + | _ | _ |
| 7 | 1 | 10 | 10 | + | _ | _ |
| $T_{MC}$ | 1 | 640 | 0 | _ | _ | _ |
| $T_{MI}$ | 1 | 0 | 10 | + | + | + |

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