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# Anti-Microbial and Anti-Fungal Activities of Methanol Extract of *Argemone mexicana* and its Potential Anti-Hepatitis Promises

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## Abstract

Hepatitis has been a major plague of mankind. The history of the discovery of causative viruses is one of the most fascinating scientific adventures of this half-century. Individualization of several types of hepatitis only emerged after world war two. Their identification has been associated with milestones which revolutionized medicine and public health. The discovery of HBV brought the first ever vaccine not prepared by tissue culture but initially directly from plasma and soon the first vaccine produced by genetic engineering. Increasing evidence suggest that Viral Hepatitis is a critical disease that kills about 15% to 25% people prematurely due to its infection. Treatment with available interferons only suppresses viral reproduction in about 40% to 90% of patients with chronic hepatitis. Most people do not have a permanent response and relapse is common; the medications do not cure the infection. Determination of zone of inhibition by means of agar well method in this study, showed that *Argemone mexicana* extract exhibited antimicrobial effects against *E. coli, B. subtilis, S. aureus*, at 25, 50, and 100 mg/ml respectively, for the antifungal activity, using *A. niger, A. fumigatus and M. species* the extracts at the same concentrations inhibited the growth of the fungi dose-dependently. The phytochemical screening of this plant revealed the presence of Tannins, saponins, flavonoids, terpenoids, alkaloids glycosides etc. which are reported to be responsible for numerous medicinal uses of plants. *A. mexicana* whose many pharmacological properties described by the traditional medical practitioners such as anti-hepatotoxic activity Anti-malarial, Antibacterial, Hepatoprotective, Anti-HIV, anti-hepatitis and in the treatment of jaundice have been confirmed by several authors hence the need for the study. It is therefore eminent for us as scientist responsible for the discovery of noble drugs to characterize this plant should we find safer potent drugs for this scourge hepatitis.

**Keywords:** *Argemone Mexicana*; Bacteria; Extract; Fungi; Hepatitis; Phytochemicals

## Introduction

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. New drugs of plant origin and new methods of producing them will continue to be an important part of the service and thus plants are considered as one of the most important and interesting subjects that should be explored for the discovery and development of newer and safer drugs [1].

*Argemone mexicana* (Papaveraceae) is commonly known as 'Mexican prickly poppy' in English and Premathandu in Tamil, "Kwallin Kura or kaki ruwan Allah" in Hausa, (class: Magnoliopsida Dicotyledons; subclass: Magnoliidae; order: Papaverales; family: Papaveraceae) it is a well-known weed in the agricultural and wastelands. [2]. The plant is self-fertile and predominantly self-pollinated that prefers light (sandy) soils, requires well-drained soil and can grow in nutritionally poor soil. It cannot grow in the shade. It requires dry or moist soil and can tolerate drought [3]. The flowers open early in the morning and last for 2-3 days. Small stingless bees are the main pollinators. It is a widely distributed plant throughout the subtropical and tropical regions of the world.

Various parts of this plant have a medicinal effect and reported to possess potent emetic, narcotic activities [4,5]. Leaves along with black pepper are used to cure diabetes leaf decoction is used in the treatment of malarial fever, inflammation and ulcers. Leaves and seeds are also reported to find application in maintaining normal blood circulation and cholesterol level in the human body and possess anti-venom property as well [5,6].

Hepatitis is a critical disease that kills about 15% to 25% people prematurely due to its infection. Treatment with available interferons only suppresses viral reproduction in about 40% to 90% of patients with chronic hepatitis. Most people do not have a permanent response and relapse is common; the medications do not cure the infection (CDC). *Argemone mexicana* is reported to have different chemical components [1]. Many pharmacological properties described by the traditional medical practitioners about *Argemone mexicana* have been confirmed by several authors such as anti-hepatotoxic activity Anti-malarial, Antibacterial, Wound healing, Antiasthmatic, Hepatoprotective, Anti-HIV, Vaso-relaxant activities, anti-hepatitis and in the treatment of jaundice [7,8]. The plant contains alkaloids as berberine, protopine, sarguinarine, optimize, chelerytherine the glycosides, terpenoids, steroids, flavonoids, reducing sugars and tannins [9]. *Argemone mexicana* is regarded as one of the most significant plant species in traditional system of medicine [10]. Hence, the need for the experimentation.

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Hepatitis is a medical condition defined by the inflammation of the liver and characterized by the presence of inflammatory cells in the tissue of the organ. Hepatitis may occur with limited or no symptoms but often leads to jaundice (a yellow discolouration of the skin, mucous membrane, and conjunctiva), poor appetite, and malaise. Hepatitis is acute when it lasts less than six months and chronic when it persists longer. According to the Centre for Disease Control and Prevention (CDC), there are 4.4 million Americans currently living with chronic hepatitis. Many more people don't even know that they have it (Figure 1).

Viral infections of the liver that are classified as hepatitis include hepatitis A, B, C, D and E. Hepatitis A is a milder version of the disease, and hepatitis C and D are more severe. Treatment options vary depending on what form of hepatitis you have and what caused the infection. You can prevent some forms of hepatitis through immunizations or lifestyle precautions.

**Figure 1:** Source: A. mexicana got from Zuru, Kebbi Stata, Northern Nigeria.

## The Aims and Objectives of the Study

The aim of this work is to compare the antibacterial and antifungal properties of *A. mexicana* (L).

### The specific objectives are

- To determine the phytochemicals present in *A. mexicana* (L).
- Antibacterial activity.
- Antifungal activity.
- Compare the results of the anti-fungal and antibacterial activities.

### The justification for the study

The justification for this study is based on the facts that

*A. mexicana* promises to be a safe and potent drug for bacterial and fungal diseases which are endemic in Africa. Traditional medical practitioners have tried it in patients with obvious hepatitis and jaundice successfully and even these claims have been confirmed by numerous scientists.

## Materials and Methods

### Plant collection and identification

The plant was collected from Zuru, Kebbi State Nigeria, identified by a taxonomist Dr D. Singh, Department of Biological Sciences, Kebbi State University, and was given (Voucher number of 152) and a specimen was deposited for future reference.

### Growth media and test microorganisms

Mueller Hilton agar was used for the antibacterial activity, while Potato Dextrose agar was used for the antifungal activity using the following organisms (*Escherichia coli, Bacillus subtilis*, *Staphylococcus aureus*), and (*Aspergillus niger, Aspergillus fumigatus* and *mucu species*) respectively obtained from Federal Medical Centre Birnin Kebbi.

### Preparation of plant extracts

The procedure described by Prabhat et al. was adopted for the preparation of a methanolic extract of *A. mexicana* Linn. A sample of the shade-dried plant of *Argemone mexicana* was made into powder using pestle and mortar. 80 g of plant powder was extracted with 500 ml of Methanol for 72 hours by Soxhlet extractor. The extract was concentrated in a water bath, transferred into pre-weighed sample containers and stored at 4°C until about the time of experiments; phytochemical screening, antibacterial and antifungal activities respectively.

### Phytochemical screening

Preliminary phytochemical screening for alkaloids, tannins, saponins, terpenoids steroids anthraquinones cardiac glycosides flavonoids and reducing sugars of methanol extracts of the whole plant were made by following standard procedures, in order to determine the secondary constituents of the plant [11].

### Antibacterial activity

Antibacterial activity was determined by cup diffusion method on Mueller Hinton agar medium.

- The Mueller Hinton agar medium will be prepared according to the manufacturer's description
- The prepared agar medium will be left standing for 24 hours after sterilization with the autoclave to be sure the agar was not contaminated.
- The sterile medium (20 ml) was poured into a 9 cm Petri plates.
- The medium was allowed to cool in a sterile condition and plates were then inoculated with cultures of test bacteria.
- Agar cup of 5 mm diameter was made in the plates with the help of sterile borers.
- The desired different concentrations of 25, 50, and 100 mg/ml of the extracts were prepared from the stock solution which is prepared

by weighing 1 g of the extract into a measuring cylinder and make it up to 10 ml of distilled water.

- A 100 µl volume of each dilution was introduced in triplicate wells into Nutrient agar plates already seeded with the standardized inoculums of the test bacterial cells.
- All test plates were incubated at 37°C for 24 hours.
- Ciprofloxacin was used as a positive reference to determine the sensitivity of each bacterial species tested.

### Antifungal activity

Antifungal activity was determined by cup diffusion method on potato dextrose agar medium.

- The Potato dextrose agar medium will be prepared according to the manufacturer's description
- The prepared agar medium will be left standing for 24 hours after sterilization with the autoclave to be sure the agar was not contaminated.
- The sterile medium (20 ml) was poured into a 9 cm Petri plates.
- The medium was allowed to cool in a sterile condition and plates were then inoculated with cultures of test fungi.
- Agar cup of 5 mm diameter was made in the plates with the help of sterile borers.
- The desired different concentrations of 25, 50, and 100 mg/ml of the extracts were prepared from the stock solution which is prepared by weighing 1 g of the extract into a measuring cylinder and make it up to 10 ml of distilled water.
- A 100 µl volume of each dilution was introduced in triplicate wells into potato dextrose agar plates already seeded with the standardized inoculums of the test fungal cells.
- All test plates were incubated at 37°C for 24 hours.
- Ketoconazole was used as a positive reference to determine the sensitivity of each fungal species tested.
- Clear zones within which fungal growth was absent were measured and recorded as the diameter (mm) of complete growth inhibition.

## Results

The phytochemical screening reveals the presence of secondary metabolites such as flavonoids, saponins, tannins, steroids, terpenoids, alkaloids, cardiac glycosides and anthraquinones, which could be responsible for the multiple medicinal implications of this plant like analgesia, anti-fungal, anti-microbial, in the treatment of jaundice and even anti-viral (Table 1).

| Parameters | Tests | Results |
|---|---|---|
| Flavonoids | NaOH test | + |
| Saponins | Frothing test | + |
| Tannins | Ferric chloride test | + |
| Steroids | Lieberman's test | + |
| Terpenoids | Salkowski test | + |
| Alkaloids | Dragendorrf's test | + |
| Reducing sugar | Fehling's test | - |
| Cardiac glycosides | Killer-Kiliani's test | + |
| Anthraquinones | Chloroform layer test | + |

**Table 1:** Result of phytochemical screening of methanol extract of *A. mexicana* (L) plant. + =Positive; - =Negative

### The result of antimicrobial activity

Table 2 which shows that *Argemone mexicana* extracts exhibited a statistically significant antimicrobial activity against the tested microbes' dose-dependently ($p<0.05$) when compared with the control (normal saline) group, through determination of zone of inhibition by means of agar well method. The lowest dose (25 mg/ml) however, did not show any statistically significant difference ($p>0.05$) when compared to the control. The standard drug ciprofloxacin 500 mg showed a statistically significant difference ($p<0.05$) when compared to the extract and the control probably because of its purity. The results are presented as mean ± SD using ANOVA.

| Zone of Inhibition in millimetres | | | | |
|---|---|---|---|---|
| **Infection** | **Treatment groups** | ***S. aureus*** | ***E. coli*** | ***B. subtilis*** |
| - | Normal saline | 0.00 | 0.00 | 0.00 |

| | | | | |
|---|---|---|---|---|
| + | Arg. 25 mg/ml | 1.00 ± 0.00 | 2.00 ± 0.00 | 0.33 ± 0.58 |
| + | Arg.50 mg/ml | 3.33 ± 1.16* | 4.67 ± 1.16* | 3.67 ± 0.58* |
| + | Arg. 100 mg/ml | 7.33 ± 0.51* | 8.67 ± 0.58* | 7.78 ± 1.168* |
| + | Cpx 500 mg/ml | 16.00 ± 2.00* | 18.00 ± 2.00* | 18.67 ± 1.53* |

**Table 2:** Antibacterial activity of *A. mexicana* methanolic extracts. * = significant zone of inhibition ($p<0.05$) when compared to the controls. Cpx = Ciprofloxacin, *S. aureus = Staphylococcus aureus, E. coli = Escherichia coli, B. subtilis = Bacillus subtillis*,

Table 3 shows the activity of the plant extract when compared to a standard anti-fungal drug. *A. mexicana* extract exhibited statistical significant difference ($p<0.05$) antifungal effects against the tested organisms when compared to the control group (normal saline). However, the standard drug showed the statistically significant difference ($p<0.05$) when compared with the extract of *A. mexicana*. In the same vein, the lowest dose did not show any statistically significant difference ($p>0.05$) when compared with the higher doses of the crude extracts. Ketoconazole (300 mg) a standard drug exhibited the best activity probably because it is a pure compound which makes it pharmacologically different from the crude extract.

| **Zone of Inhibition in millimetres** | | | | |
|---|---|---|---|---|
| **Infection** | **Treatment groups** | ***A. niger*** | ***M. spp*** | ***A. fumigatus*** |
| - | Normal saline | 0.00 | 0.00 | 0.00 |
| + | Arg. 25 mg/ml | 0.33 ± 0.58 | 0.00 ± 0.00 | 1.33 ± 0.57 |
| + | Arg.50 mg/ml | 2.33 ± 0.57* | 1.333 ± 0.58 | 3.00 ± 0.00* |
| + | Arg. 100 mg/ml | 5.67 ± 0.57* | 3.333 ± 0.57* | 3.00 ± 0.00* |
| + | Ktx 300 mg/ml | 9.00 ± 1.00* | 7.33 ± 1.16* | 9.67 ± 0.58* |

**Table 3:** Antifungal activity of *A. mexicana* methanolic extract. * = significant zone of inhibition ($p<0.05$) when compared to the controls. Ktx=Ketoconazole, A. *niger =Aspergillus niger, M. spp = Mucu species, A. fumigatus = Aspergillus fumigatus*

## Discussion and Conclusion

The results of these work show that the plant contains both antibacterial and anti-fungal properties; Table 2 shows that the higher doses (100 mg/ml and 50 mg/ml) of the crude extracts of *A. mexicana* showed statistically significant antimicrobial activity ($p<0.05$) when compared with the control group (normal saline). This is possibly due to the phytochemical constituents present in the plant extract. *A. mexicana* is reported to have different chemical components [1]. Many pharmacological properties described by the traditional medical practitioners about *A. mexicana* have also been confirmed by several authors such as anti-hepatotoxic activity Antibacterial, Wound healing, Hepatoprotective, Anti-HIV, Vaso-relaxant activities, anti-hepatitis and in the treatment of jaundice [7,8]. This could be due to the fact that the plant contains secondary metabolites like; alkaloids as berberine, protopine, sarguinarine, optimize, chelerytherine, cardiac glycosides, terpenoids, steroids, flavonoids, reducing sugars and tannins which have been reported to have these activities [9]. The lowest dose, however, (25 mg/ml) did not show any significant antibacterial activity ($p>0.05$) when compared to the control, this could be due to the fact that the dose is too small to elicit any activity on the microbes because of the crude nature of the extract. Methanolic extracts of *A. mexicana* have been reported to show inhibitory activities in a large number of organisms; [12,13] reported the antibacterial activities and diameter of the zone of inhibition of A. Mexicana to be higher than that of *O. gratissimum*, Syzygium, and aromatic seed, cinnamon bark, *S. officinalis* and several others. These are in agreement with the findings in this work which showed significant antibacterial and anti-fungal activities ($p<0.05$) of the methanol extracts of the leaves of this wonder plant whose literatures and personal discussions with traditional medical practitioners confirmed that infusion of the leaves of this plant can cure jaundice, hepatitis (even though there is no data in this work) and several opportunistic infections (Livestock clinic, Zuru Local Government Kebbi State 2007). The plant is confirmed to inhibit the growth of *Brucella abortus* which is the most common cause of abortion among domestic livestock. Hence our recommendation for *in vivo* studies using animal cell lines impregnated with hepatitis B and C viruses respectively, in order to establish these claims and many more, as well as to characterize the extracts of *A. mexicana* towards identifying the active ingredients responsible for these numerous antibacterial, anti-fungal even anti-viral properties.

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