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Antibacterial Activity of *Argemone mexicana* Seeds Extract Against *Staphylococcus aureus* and *Escherichia coli*

Ekwal Imam * [1], Zewdneh Tomass [1], Hafte Assefa [1] and Addisie Yalew [1]
[1] Department of Biology, College of Natural and Computational Science, Mekelle University, Mekelle, Ethiopia

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Abstract: Antibacterial activity of ethanolic extract of *Argemone mexicana* seeds was assessed against *Escherichia coli* and *Staphylococcus aureus* by disc-diffusion method. The extract had significant inhibitory effect against *Staphylococcus aureus* and *Escherichia coli* with Minimum Inhibition Concentration values of 3 mg/ml and 3.5 mg/ml respectively. The phytochemical analysis of *A. mexicana* seeds demonstrated that the secondary metabolites such as tannins, flavonoids and steroids were present. The antibacterial activity of *A. mexicana* seeds extract may be due to such secondary metabolites.

Key words: *Argemone mexicana*, antibacterial activity, disc diffusion method, Mueller.

Introduction

Medicinal plants represent a rich source of antimicrobial agents and are used as raw materials in medical and veterinary drug industries [34]. A wide range of medicinal plant parts are used as raw drugs because they contain various medicinal ingredients. Parts of plants used for medicinal purpose include roots, stems, flowers, fruits, twigs and modified plant organs. Some of the plant parts which are used as raw drugs are collected in smaller quantities by the local communities and folk healers for local use, whereas many other plant origin raw drugs are collected in larger quantities and traded as raw materials for herbal industries [36]. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated [3]. The potential of higher plants as sources for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated for phytochemicals and the fraction analyzed for biological or pharma-cological activities is even smaller [9].

Historically, pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as a tool in discovering new biologically active molecules has been most productive in the area of antibiotics [12,19]. Even now, contrary to common belief, drugs from higher plants continue to occupy an important niche in modern medicine. On a global basis, at least 130 drugs, all single chemical entities have been extracted from higher plants, or modified further synthetically are currently in use, though some of them are recently made synthetically for economic reasons [24]. The genetic ability of pathogenic bacteria to develop resistance against commonly used antibiotics is a major public

*Corresponding author (Ekwal Imam)
E-mail: < ekwalimam01@gmail.com >

health and veterinary challenge worldwide, posing a big threat to the wellbeing of human and domestic animal population [11,22,39]. This has necessitated a search for alternative antibacterial substances from various natural sources, including flowering plants.

A number of studies have been conducted on the identification of crude plant extracts in a therapeutic treatment of bacterial infections [7,15,18]. The discovery and development of structurally novel chemical entities to control the multi-drug resistant pathogenic bacteria is desperately desired by the pharmaceutical industries and drug developers, who are working towards the under explored natural sources for developing the front line drugs. The use of the plant extracts and phytochemicals can be of great significance in therapeutic treatments and could be helpful to control multi-drug resistant pathogenic microorganisms. For example, the growth of the multi-drug resistant *Pseudemons aeruginosa* was inhibited by the extracts from *Caryophyllus aromatic* (clove), *Syzygyumjo abolanum* (jambolan), *Punica granatum* (pomegranate) and *Thymus vulgaris* (thyme) [21]. World Health organization (WHO) has also advocated that the medicinal plants would be the best source of obtaining a variety of drugs [4]. A number of plant secondary metabolites like alkaloids and flavonoids have been used as antiviral, antibacterial, antiamoebal and anticancer agents [2,16,17,31,32]. The glycoside and saponins from *Quillaja saponaria* and *Acacia auriculoformis* were found to be antiprotozoal *in vitro* [38]. Phenolics and polyphenols are the other group of plant secondary metabolites that exhibit antimicrobial activity. Flavonoid synthesized by plants in response to the microbial infection, have antimicrobial activities against a wide array of microorganisms [6].

Argemone mexicana has numerous medicinal values and throughout the tropic, widely used as a medicinal plant. It contains numerous isoquinoline alkaloids. The total alkaloid fraction in the dried roots and stems is 0.25 %, mainly consisting of protopine or berberine. Protopine and sanguinarine showed molluscicidal properties against *Lymnaea acuminate* and *Biompharia globrata*, whereas alkaloid fraction from the roots showed anti-inflammatory activity in rabbits and rats [14]. Its seed contains 35-40 % of orange yellow oil which consists mainly of linoleic acid and oleic acid [23]. *Argemone mexicana* is widely used in traditional medicine because it is considered as pain killer, diuretic, cholagogue and anti-inflammatory. The seed oil is used as a purgative and as pomade. Both the seed oil and leaf infusions are drunk to relieve cough; root and leaf decoctions are applied to the skin to cure edema, inflammation, muscle pain, ulcers and yaws. Leaf sap is used as eardrops to cure ear inflammation. Flowers, leaves and seed are used in drinks for their psycho-active properties. In Nigeria, the seed oil is applied to protect wood from termite attack, whereas in India, Mexico and the West Indies the seed oil is sometimes used to make scup, for greasing and illumination [7].

Countries like Ethiopia, where, people are using medicinal plants products traditionally, have little information about antimicrobial activity of some important medicinal plants. Except few [7], no adequate information is available on *in vitro* antimicrobial activity of *A. mexicana* against bacterial pathogens. This study is a small effort in which antimicrobial activity of ethanolic seeds extracts of *A. mexicana* was evaluated against *Staphylococcus aureus* and *Escherichia coli*.

Methodology

Plant material collection and extraction

The study started with collection of seed bearing healthy plants of *A. mexicana*. The fresh seeds were collected from the plants and washed thrice with distilled water and dried using blotting paper in the laboratory. Subsequently seeds were grinded using mortar and pestle.

Crude seed extract of *A. mexicana* was prepared using maceration technique following Rahber [27]. Hundred grams of dried powder of seeds was mixed with 100 ml of 96 % ethanol in a conical flask and was kept at 25°C for 72 hours. The suspension was filtered through a Whatman filter paper and subjected to rotary evaporator to obtain the extract. The extract obtained was stored at 4°C until used.

Test bacteria

Bacterial cultures of *Escherichia coli* and

Staphylococcus aureus were obtained from the culture collection center of the Department of Veterinary Medicine, College of Veterinary Medicine, Mekelle University, Ethiopia. The bacteria were maintained on nutrient agar at 37°C for 24 hours.

Antibacterial activity

The ethanol seed extract of *A. mexicana* was tested by the modified Kirby Bauer disk diffusion susceptibility method [5]. The bacterial strains (4-5 colonies) to be tested were suspended in 4 ml of sterile normal saline (0.85 %). The surface of the sterile Mueller Hinton agar (MHA) in the Petri dishes was dried and the test bacteria were inoculated separately with a sterile swab to obtain a bacterial lawn.

From seed extract, 0.5 gram was mixed with 5 ml (100 mg/ml), 2.5 ml (200 mg/ml), 1.67 ml (300 mg/ml) and 1.25 ml (400 mg/ml) of distilled water and aseptically transferred to sterile paper disks (6 mm diameter) made of a Whatman no 1 filter paper. The extract impregnated disks were placed to Mueller Hinton agar. After incubation of plates for 24 hours at 37°C, the diameter of the inhibition zones was measured in millimeters (mm). Sterile distilled water was used as positive control in each plate. Plates prepared using the same procedures without extract were equally set as negative controls.

Phytochemical composition

Seed extract of *A. mexicana* was also tested for tannin, flavonoid and steroid properties. The presence of tannins and flavonoids was performed according to Trease and Evans [35]. The presence of steroids was determined according to Sofowora [33].

Probit analysis for Minimum Inhibition Concentration

Minimum Inhibition Concentration (MIC) is the least concentration of the extract of *A. maxicana* at which zone of inhibition can be seen clearly. Probit analysis is a statistical method by which MIC can be analysed easily. Probit analysis is a type of regression used to analyze binomial response variables. It transforms the sigmoid dose-response curve to a straight line that can then be analyzed by regression. It is used to analyze many kinds of dose-response or binomial response experiments in a variety of fields. This is done by testing the response of an organism under various concentrations of each of the chemicals in question and then comparing the concentrations at which one encounters a response. Once a regression is run, output of the probit analysis can be used to compare the amount of chemical required to create the same response in each of the various chemicals. There are many endpoints used to compare the differing toxicities of chemicals, but the LC_{50} (liquids) or LD_{50} (solids) are the most widely used outcomes of the modern dose-response experiments. The LC_{50}/LD_{50} represent the concentration (LC_{50}) or dose (LD_{50}) at which 50 % of the population responds. Similarly this method can also be used to analyze Minimum Inhibition Concentration (MIC) for an antibacterial substance against microorganisms. In this study SPSS 16.0 is used to run probit analysis.

Results and discussion

Potency of antibacterial activity of phytochemicals is affected by multiple factors ranging from agro-ecological condition of the locality where the plant grows to the type of solvent used for extraction of the chemicals. In this study we preferred to use ethanol as our extraction solvent because of its relative accessibility. Furthermore, communities living in rural areas of Ethiopia commonly use locally made alcoholic drink known as "Tela" or "Areke" as solvents to prepare traditional plant origin remedies against human as well as veterinary ailments. The most commonly used solvents for investigation of antimicrobial activity in plants are methanol, ethanol and water [8,20,26,29]. The disc-diffusion method for antibacterial activity showed strong reduction in bacterial growth in terms of zone of inhibition.

The antibacterial activity of crude seed extract of *Argemone mexicana* against *E. coli* and *S. aureus* with different concentration is shown in Fig.1, whereas Minimum Inhibition Concentration (MIC) was calculated for both microbes

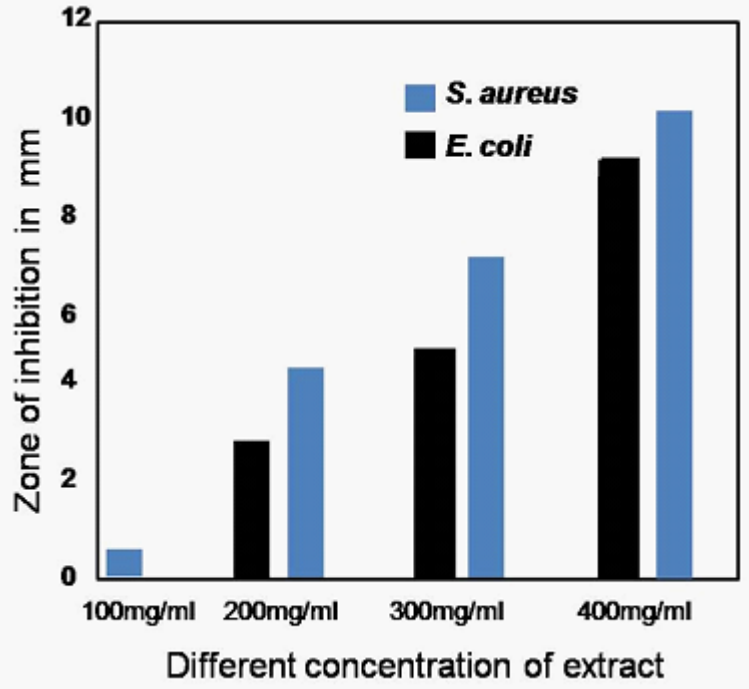

Fig. 1. Antibacterial activity of crude seed extract of *Argemone mexicana* against *E. coli* and *S. aureus* with different concentration

and it was recorded 3 mg/ml for *Staphylococcus aureus* and 3.5 mg/ml for *Escherichia coli* respectively. It was also observed that zone of inhibition increased with increase in number of days for both bacterial species. When the concentration of the extract was lowered from 400 mg/ml to 100 mg/ml, slight decline in the inhibition zone was shown by the ethanol extract of the seed of *A. mexicana*. At the first day the inhibition zone of *E. coli* was 0 mm (day-1), 1 mm (day-2), 2 mm (day-3), 5 mm (day-4) at the same concentration of 100 mg/ml but in day 4 inhibition zone was increased to 9 mm (day-l), 11 mm (day-2), 13 mm (day-3) and 19 mm (day-4) in concentration level of 400 mg/ml, respectively (Fig. 2).

These changes in inhibition zone occurred due to continuous action of the extract residue on the bacterial cells at various level of concentrations. In plants the inhibitory substances are produced as a result of enzyme action and degree of stability of the active substances during the wilting and drying of the plant also varies greatly. The power of inhibition depends on the timing; some of the plants extract show results when pathogens are treated for longer period, whereas, in some of the cases, plants extract begin to lose the power of inhibition in very short period of time [25]. Similar phenomenon is also observed by other researchers [10,30]. When tested by the disc diffusion method, the ethanol seed extract of *A. Mexicana* showed strong antibacterial activity against *S. aureus* with zone of inhibition of 15 mm, which is an indication of strong antibacterial activity as it is suggested that an inhibition zone of 14 mm or greater (including diameter of the disc) can be considered as high antibacterial activity [28]. The highest antibacterial activity of 21 mm in *S. aureus* and least was recorded in *E. coli* measured 19 mm with zone of inhibition at concentration of 400 mg/ml respectively.

The most pronounced activity with inhibition of 21 mm was observed against *S. aureus* at concentration of 400 mg/ml in day four. At concentration of 100 mg/ml, the inhibition zone was 0.5 mm (day-1), 2 mm (day-2), 4.5 mm (day-3) and 7 mm (day-4) zone, whereas, in concentration of 400 mg/ml, inhibition zones of 10 mm (day-1), 13 mm (day-2), 16 mm (day-3) and 21 mm (day-4) were recorded (Fig. 3).

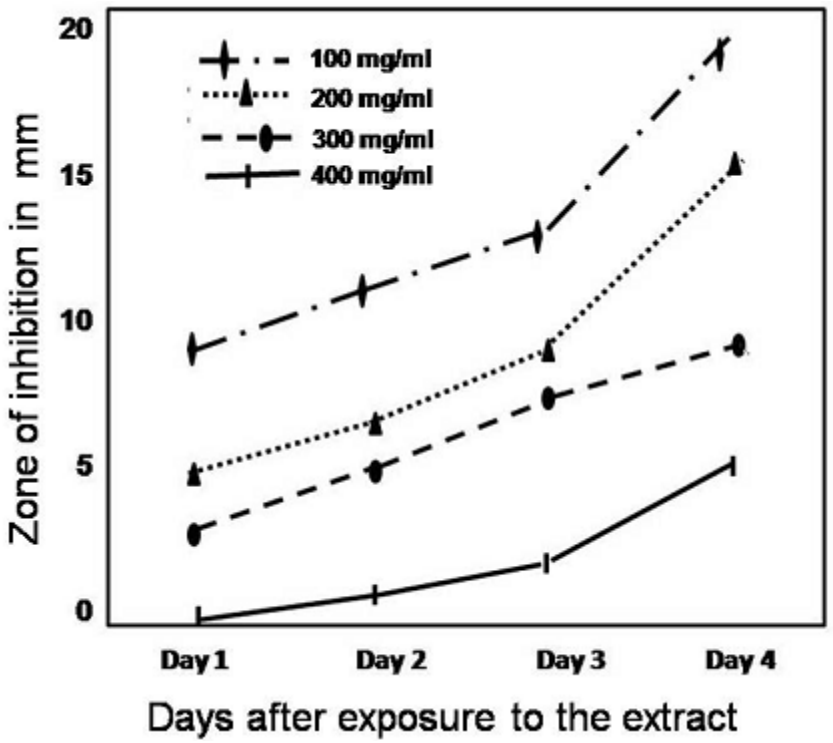

Fig.2. Evaluation of various concentrations of ethanol extract of *Argemone mexicana* with inhibition zone against *E. coli* with different days

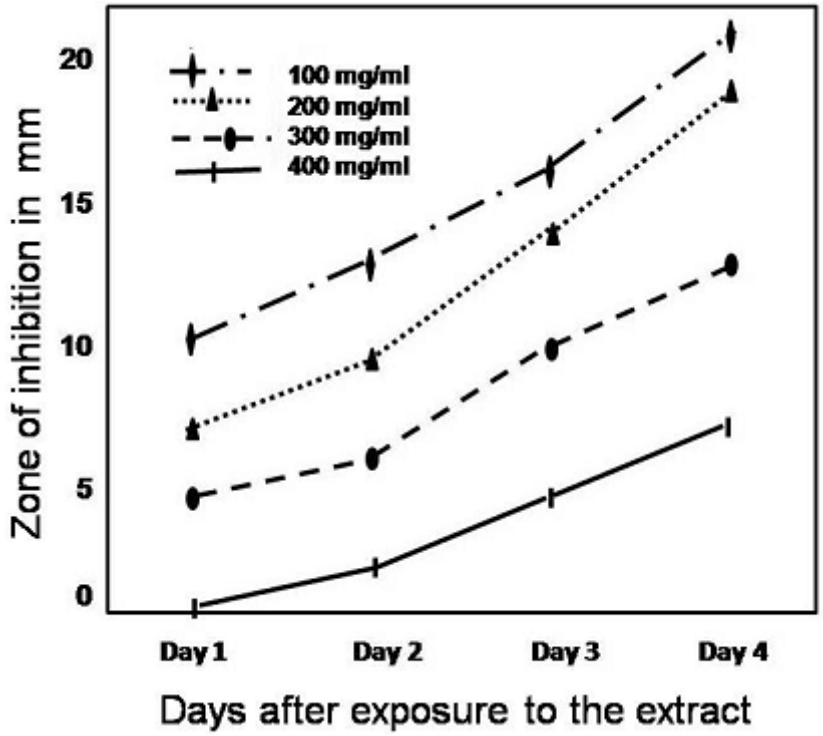

Fig. 3. Evaluation of various concentrations of ethanol extract of *Argemone mexicana* with inhibition zone against *S. aureus* with different days

A number of plant secondary metabolites like alkaloids and flavonoids have been reported as anticancer, antiviral, antibacterial and antiamoebal agent [16,17,31,32]. While glycosides and saponins have antiprotozoal activity of *A. mexicana* revealed the presence of labdane type diterpene compounds and these compounds are similar to those that have been reported to possess strong antibacterial activity [1]. It is likely that the presence of diterpene may have contributed to the antimicrobial activity of seed extract of *A. mexicana*. These classes such as tannins, flavonoids and steroids of the compounds are known to have curative activity against several pathogens and therefore could suggest tradition use for the treatment of various diseases [13]. The broad antimicrobial activities of this extract could be as a result of the plant secondary metabolites (such as Tannins, Steroids, and Flavonoids) present in the seed of *A. mexicana.* In line with these findings, Usman and Osuji [37] reported that tannins had been widely used typically to sprains, bruises and superficial wounds as such, and it seems that presence of tannins and other plant properties were responsible for these broad activities. The development of blue-black colour while doing tannin compound test confirmed the presence of tannin in *A. Mexicana*. Similarly the color change of orange and blue indicates the presence of flavonoids and steroids respectively. The phyto-chemical active compound of *A. mexicana* were also recorded, however it needs standardization (Table 1).

Table 1. Preliminary phytochemical screening of seed powder of *Argemone maxicana*

Test group	Seed extract
Flavonoids	+
Tannins	+
Steroids	+

Conclusion and recommendation

A. mexicana exhibits excellent potential of antibacterial activity againts *Staphylococcus aureus* and *Escherichia coli* revealed that some of the medicinal plants used in traditional medicine are potentially effective as antibacterial agents.

The present study is an *in-vitro* antibacterial evaluation of a plant and it forms a primary platform for further phytochemical studies. However, it recommends that further standardized research is necessary to determine the identity of the antibacterial compounds from different plants and also to determine their full spectrum of efficacy. Exploration of plant-derived antimicrobials is a need to day because plant based antibacterial activity have enormous therapeutic potential and can serve the purpose with lesser side effect that are often associated with synthetic antimicrobials. Awareness is also needed for developing antibacterial activity from higher plant, which may be rewarding as it will lead to the development of a phyto-medicine at commercial level to act against many microbes.

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