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Original article

In-vitro antibacterial and antifungal properties of the organic solvent extract of *Argemone mexicana* L.

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ABSTRACT

Plants have been reported showing strong antimicrobial properties. Investigation of plants originated antibiotic agents explored a new chapter in the field of pharmacology. This study was designed to screen *Argemone mexicana* L. for its antibiotic properties and its positive effect against pathogenic bacteria and fungi. Flowers, berries and leaves were extracted from three solvents (methanol, ethanol and chloroform) and Antibacterial and antifungal activates were performed through agar disc diffusion and agar tube dilution methods, respectively. Antibacterial activity of ethanol, methanol and chloroform was about 80% against maximum bacterial strains. Antifungal activity was observed more than 50%. Crude plant extracts were potent 50–60% against *A. fumigates* while above 75% against *A. niger*.

1. Introduction

Utilization of plants as drug ranges as of 4–20% in various countries as well as concerning 2500 species is sold globally (Schippmann et al., 2002; Al-Dhabi et al., 2015; Antonisamy et al., 2015; Al-Dhabi and Valan Arasu, 2016; Arokiyaraj et al., 2015; Balamurugan, 2015; Barathikannan et al., 2016; Cuong et al., 2017; Elango et al., 2017). Therapeutic plant lives have been used by local healers and in traditional drugs, 80% of the people live in villages and cities are mostly reliant in Unani method of medicine (Ikram and Hussain, 1978; Elango et al., 2016a; Fowsiya et al., 2016). Indigenous medicinal plants are rich source of active constituents that can act as anti-microbial agents (Glorybai et al., 2015;Elango et al., 2016b; Gurusamy et al., 2019). Across the world, plants have been used as medicines and are found better in results to cure specific (Haritha et al., 2016; Helan et al., 2016; Ilavenil et al., 2017). Numerous plant species and their parts have been used as raw plant material to extract medicines and they course wide-ranging medicinal stuffs. Reported plant parts that have used as active drugs includes flowers, stem, fruits, seeds, barriers, leaves, bark, root, latex and other modified organs (Al-Nafie, 2008: Mahmood et al., 2012a; Kannan and Agastian, 2015; Park et al., 2016a). Local community collect mostly use a small quantity of raw plant material as drug to treat disease. Raw plant material is also collected at large scale and these drugs/plant parts are traded/sold to the market for economic benefits (Uniyal et al., 2006; Park et al., 2016b). Plant species are being evaluated for biological activities. Hundreds of them have been investigated for biological activities. However a huge mainstream of them have not been effectively investigated (Liu and Wang, 2008; Rathi et al., 2015; Park et al., 2017; Rajkumari et al., 2019).

Plants have been reported showing strong antimicrobial properties (Surendra et al., 2016a; Surendra et al., 2016b; Surendra et al., 2016c). Investigation of plants originated antibiotic agents explored a new chapter in the field of pharmacology (Valsalam

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et al., 2019a; Valsalam et al., 2019b). Various plant species are used science antiquity against skin diseases for example extract of *Aloe vera* is very caring for dandruff that is a fungal disease and this plant has strong antifungal properties. Looking ethnobotanical studies a number of plants are screened for antimicrobial activities and they showed positive results. To cure microbial disorders pharmaceutical companies are paying interest to discover new medicines from plant origin as they are safe to use and less expensive and will be in approach to population(Gerhartz et al., 2005). Synthetic medicines against dermatophytes are somehow expensive. In this research work antibacterial and antifungal activity was performed on the most common plants that are in easy approach to everyone, to investigate the antibiotic agents, which may be helpful for pharmaceutical companies to design a less expensive medicine (Haq, 1997).

Fungi are responsible for many skin diseases like ringworm and dandruff. Dermatophytes causing these diseases reside in the tropical and subtropical areas. Fungi that cause skin problems mostly live in the upper top layer of skin, dead parts of skin, and moist areas of body, under arms and under breasts. Normally fungi living in such places cause minor infections that are easily curable. On the other hand fungi can cause serious infectious diseases that can be more hazards. They can penetrate into skin and cause itching, swelling and other such types of symptoms to body. Fungal infection at one place of the body can show its reaction to elsewhere for example a person suffering from infection between toes may develop a reaction on the fingers or hands after contacting to that part. The dermatophytes, *Microsporum canis, Trichophyton, Epidermophyton* are mostly responsible for such types of infectious problems. Clinical differentiation of such dermatophytes is not an easy task. To treat such diseases a special clinical care is required by a physician (Beentje, 1994). This study has been designed to screen *Argemone mexicana* L. a member of family papaveraceae for its antibiotic properties and its positive effectsagainst pathogenic bacteria and fungi.

2. Materials and methods

2.1. Plant used for bioassays

Argemone mexicana L belonging to Papaveraceae family was selected for bioassays (antimicrobial activities) extracted from three solvents (methanol, ethanoland chloroform). Flowers, berries and leaves were collected for aforementioned activities.

2.2. Plant material extraction and dose preparation

Plant parts were collected and transported to laboratory, where they were cleaned and washed with distilled water and dried with blotting paper and finally shad dried for seven days. Exposure of sunlight was avoided to restore any kind of active volatile constituent of plant part. Drying was subjected at 20 °C and complete dried plant was grinded into powder form for further analysis.

Analytical grade solvents (methanol, ethanol and chloroform) were purchased from MERCK (Germany). Powder form of plant material was soaked for a week in respective solvent and stirred on daily basis to achieve complete extraction of active plant substances. After seven days extract was subjected to filtration (Whatman 42 filter paper), where three time filtration was done with respective solvent on each time. Filtrate was concentrated through rotary evaporator at 40 °C and a semisolid crude extract was achieved. Semi-solid filtrate of crude extract was allowed for complete drying at room temperature. Complete dry crude extract was then stored at −4 °C and used as per requirement for dose preparation in DMSO (dimethyl sulphoxide) for anti-microbial activities (Barrett and Kiefer, 1996). Detailed methodology of dose preparation has been presented in Supporting Information table 1.

Stock solution was prepared by DMSO, as this solvent has been reported to have no effect on bacteria and fungi (Fatima et al., 2009). Doxycycline (DOX), a standard antibiotic was used as positive control for antibacterial activities. Terbinafin was used as positive control for antifungal activities, while DMSO was used as negative control dose. 100 µl plant material, 100 µl DMSO and 83 µl of terbinafin was used for experimentations (Fatima et al., 2009).

2.3. In-vitro anti-microbial bioassay

Antibacterial and antifungal activates were performed through agar disc diffusion and agar tube dilution methods, respectively (Arulvasu et al., 2010; Mahmood et al., 2012).

Two gram negative bacterial strains (*Staphylococcus aureus* and *Bacillus subtilis*) and four gram positive bacterial strains (*Escherichia coli*, *Klebsiellapneumonia, Vibrio cholera* and *Enterobacter aerogenes*) were used for anti-bacterial activities. Antifungal activities were performed against two dermatophytes *Aspergillus fumigates* and *Aspergillus nigar.*

Disc diffusion method was used for anti-bacterial assays, where agar plats were allowed to solidify and then agar was punched with sterile cork borer. Agar plats were seeded with bacterial strains and a dose of 80 µl test solution was introduced to wells. Petri plats were incubated for 24 h at 37 °C.

Agar tube dilution method was used for antifungal activities. Agar was prepared and dose was inoculated in melted agar with respective quantity. Agar was then allowed to solidify and fungi were inoculated; finally test tubes were placed in incubator at 37 °C overnight.

2.4. Data and statistical analysis

Microsoft excel software (version 13) was used for basic descriptive statistical analysis.Linear growths (LG) for antibacterial activities and antifungal activities were calculated through measuring the inhibition zones diameter in millimeters. Antifungal activities were measured by formula described by Mahmood et al., 2012. Data was analyzed by one way ANOVA with 95% level of confidence ($P < 0.05$).

3. Results

3.1. Antibacterial bioassay

Results of anti-bacterial activities are provided visually in Figs. 1–4. Fig. 1 represents the experimentation with clear indication and understanding of inhibition zones, while Figs. 2–4 are actual results representations for antibacterial activities. Zones of

Table 1
Results of anti-fungal activities of methanol, ethanol and chloroform crude extract.

Solvent	Linear Growth (mm)	*A. niger*	*A. fumigates*
Methanol	Control	118	103
	Test	89	52
	% Inhibition	**75**	**50**
Ethanol	Control	112	110
	Test	90	61
	% Inhibition	**80**	**55**
Chloroform	Control	121	115
	Test	85	63
	% Inhibition	**76**	**55**

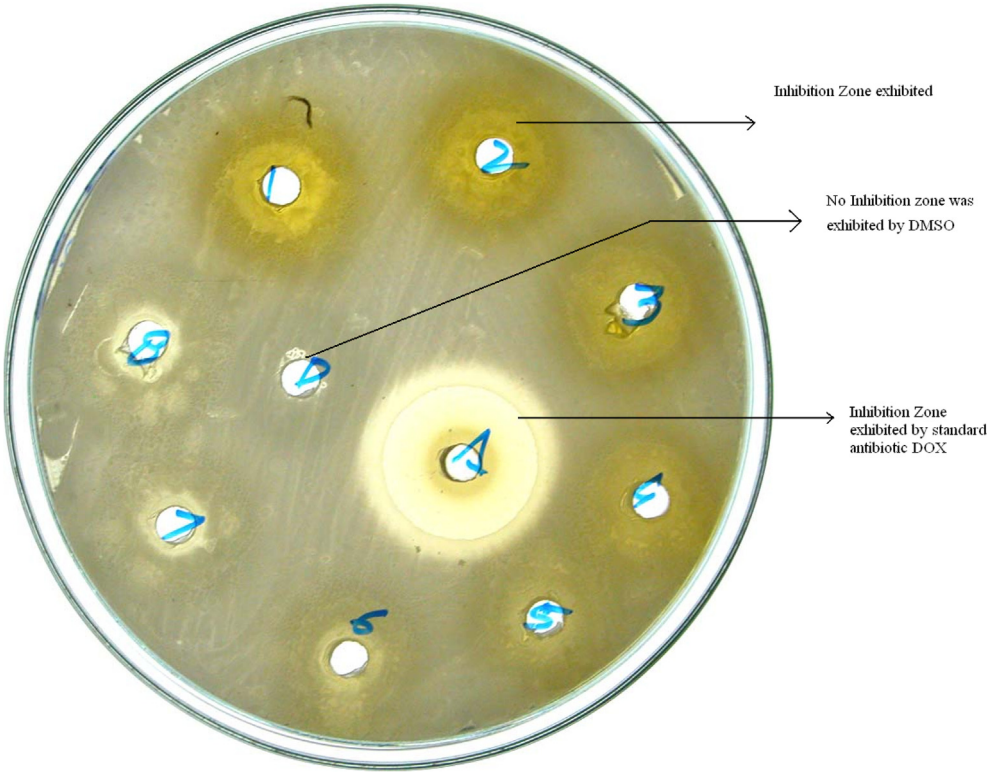

Fig. 1. Inhibition zones and crude plant extract activity observed after experiment.

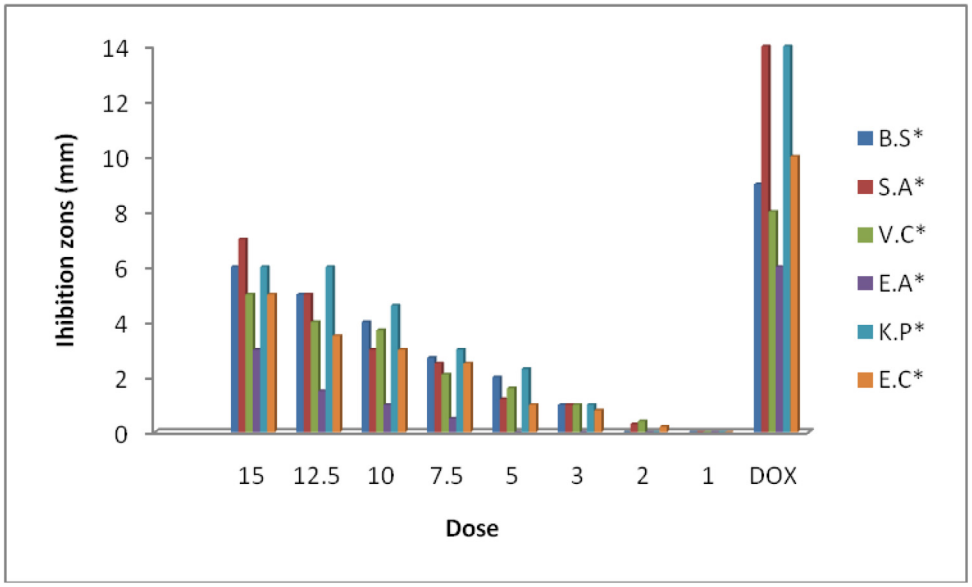

Fig. 2. Inhibitions zones (mm) against *Bacillus subtilis (B.S), Staphylococcus aureus (S.A), Vibrio cholera (V.C), Enterobacter aerogenes (E.A), Klebsiella pneumonia (K.P), Escherichia coli (E.C)*by methanolic plant extract.

inhibitions were measured in mm (millimeters) and compared with DOX for interpretation of results.

It was found that crude ethanol extract was more potent against bacteria, followed by the chloroform and methanolic crude plant extract of *A. mexicana*. Results of one way ANOVA ($P > 0.05$) also revealed the similar results and depicted a significant difference in potency of tested solvent used for extraction. It has also been observed that all three crude extracts exhibited considerable antibacterial activity, which revealed that*A. mexicana* has clear antibiotic potential. *Bacillus subtilis*was found to be least resistant to crude plant extracts followed by *Staphylococcus aureus, Vibrio cholera, Enterobacter aerogenes, Klebsiella pneumonia*and*Escherichia coli.*

3.2. Antifungal activities

Table 1 presents the descriptive statistical analysis of antifungal activities of *A. mexicana*. Visual expressions of experiment

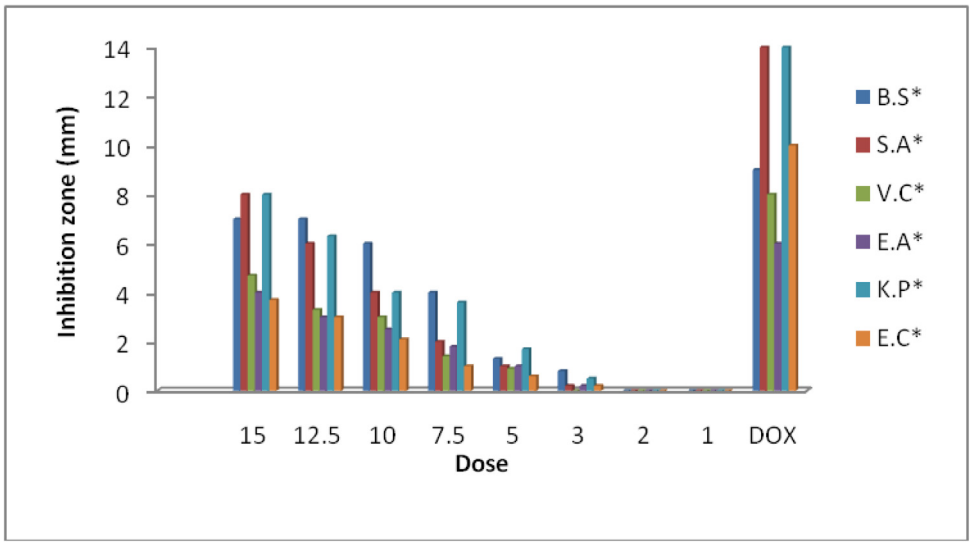

Fig. 3. Inhibitions zones (mm) against *Bacillus subtilis (B.S), Staphylococcus aureus (S.A), Vibrio cholera (V.C), Enterobacter aerogenes (E.A), Klebsiella pneumonia (K.P), Escherichia coli (E.C)*by ethanolic plant extract.

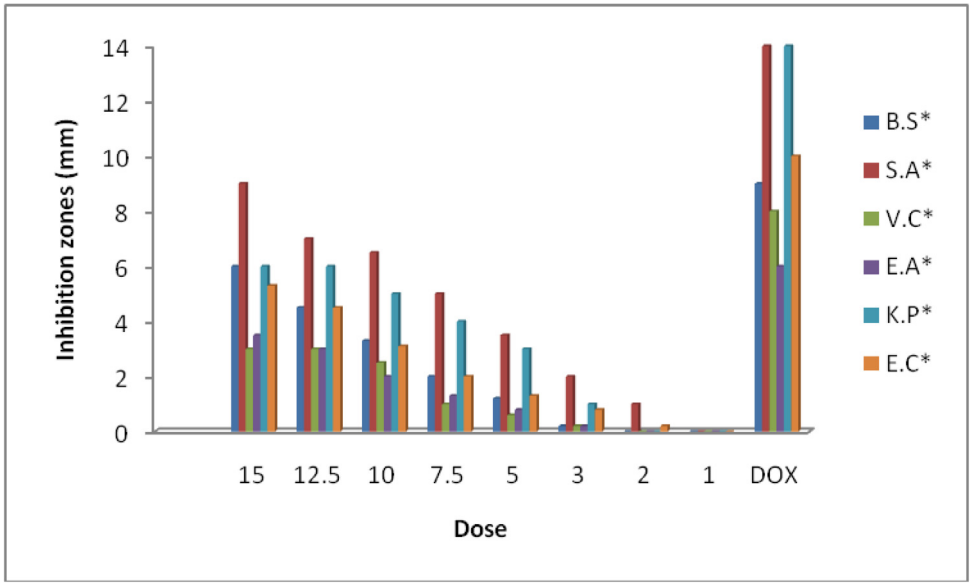

Fig. 4. Inhibitions zones (mm) against *Bacillus subtilis (B.S), Staphylococcus aureus (S.A), Vibrio cholera (V.C), Enterobacter aerogenes (E.A), Klebsiella pneumonia (K.P), Escherichia coli (E.C)*by chloroform plant extract.

performed for bio-assay have been provided in supporting information plate 1. Results of this bioassay showed that maximum antifungal activity was showed by ethanolic crude plant extract, followed by the methanol and chloroform. *A. fumigates* was found to be more resistant against plant extract dose and its inhibition was about 52% against all the solvent extracts while *A. niger*was found to be least resistant to plant crude extract and maximum antibiotic activity has been observed against this fungal strain. Overall, Ethanol has been found to be significant solvent for extraction of active plant substances, while methanol and chloroform were found to exhibit almost similar activities.

4. Discussion

Argemone mexicana L. is found in sub-tropical zone, is the member of family Papaveraceae, which is a branched annual herb with maximum of 1.3 m height. This plant is known as expectorant with wound healing abilities. Other medicinal used includes laxative and emollient agent, cure premature ejaculation and spermetoria (Mahmood et al., 2013). This plant contains some poisonous agents that provoke to use it carefully.

Results of current study were in accordance to previously published reports across the world (Mahmood et al., 2012; Mahmood et al., 2012a; Tambekar and Khante, 2010) Previously, various scientists tried to explore antimicrobial activities of plants that provided a baseline to phytochemists for new drug discoveries. Tambekar and Khante, (2010) analyzed different plant species for antibacterial activities against various bacterial strains. Plants used in the study and their parts were *Acacialeucopholia* (Bark), *Butea monosperma* (Seed, Flowers), *Woodfordia fructicosa* (Root, Flowers), *Sphaeranthus indicus* (Fruits, Whole plant), *Maytenus emerginata* (Root, Leaves). These plants samples were chosen for testing their antibacterial properties. Plant parts were dried and converted into

powdered form then extracted in different solvents like water, ethanol, methanol and acetone and antibacterial activity was tested by disc diffusion method against standard cultures of *Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes, Pseudomonasaeruginosa, Salmonella typhi, Salmonella paratyphi, Salmonella typhimurium, Klebsiella pneumoniae, Shigella flexneri*. Methanol extract of *Woodfordia fructicosa, Acacia leucopholia,* and ethanol extracts of *Sphaeranthus indicus, Butea monosperma, and Maytenus emerginata* showed strong antibacterial activity against the pathogenic bacterial strains. Phytochemical studied of the methanol extracts of *Woodfordia fructicosa, Acacialeucopholia* showed incidence of cardiac glycosides, anthraquinone, flavonoids, tannins phenolics. MIC of investigated *E. coli, B. cereus, Klebsiella* was 300 μg/mL inMethanol extract. *S. aureus* was most susceptible test pathogen to investigated plant extracts. It was sensitive to ethanol extracts. Ethanol extract of *Butea monosperma* was powerful antibacterial agent against *Sal. typhi, E. coli, Pseudomonas* and *Sal. paratyphi. Shigella flexneri* and reasonable sensitivity against *S. aureus, Ent. aerogenes, Sal. typhimurium, Pr. vulgaris* and *Klebsiella sp*(Tambekar and Khante, 2010).

Argemone mexicana has been found active plant bacterial pathogens and fungal dermatophytes. Anti-microbial bioassays of this plant were observed to be depending upon choice of solvent used for extraction of active ingredients. Method used for bioassays was easy to handle and cheap technique to predict and screen a potential of antibiotic drug discovery from any plant or natural compound. Solvent selection has been found to play a vital role in extraction of active biological compounds, which has a great influence towards new drug discovery. Our results declared that ethanol is the best solvent for extraction of active compounds from plant. These results/findings are in accordance with the previous studies from other scientists (Mahmood et al., 2012; Mahmood et al., 2012a; Tambekar and Khante, 2010). However, all the screened solvents were found to have potential for antibacterial and antifungal activities. Antibacterial activity of ethanol, methanol and chloroform was about 80% against maximum bacterial strains. Antifungal activity was observed more than 50%. Crude plant extracts were potent 50–60% against *A. fumigates* while above 75% against *A. niger*.

5. Conclusion

Current research work has explored *Argemone mexicana*potential as antibiotic drug against pathogenic microbes. Our investigations resulted good antimicrobial activities against all the tested mico-organisms. Solvent selection has also been proved good and our conclusions are in accordance with previous reports that ethanol can act as potential solvent to extract antibiotic agents. However, potent results were also observed in case of methanol ad chloroform crude extract. These positive results are the basic framework for pharmaceutical industries to pay special attention on this plant for further drugs/active constituent's exploration.

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