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ANTIBACTERIAL ACTIVITY OF COMBINED EXTRACT OF *TRIDAX PROCUMBENS* AND *ARGEMONE MEXICANA*

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ABSTRACT: Due to the indiscriminate and irrational use of antibiotics, the multidrug resistance developed especially in the hospital environment, which now becomes today's burning global issue. To overcome this problem various scientific communities are engaged in search of drugs that becomes potent antimicrobial agents. Tremendous number of medicinal drugs has been isolated from natural resources**.** One of the best natural resource is plant. Two of such plants, *Tridax procumbens and Argemone Mexicana* belongs to the family Asteraceae and family Papavraceae were taken for study.The endeavor of present study is to test the antibacterial potential of *Tridax procumbens* and A. *mexicana against* Gram positive and Gram negative bacteria. The antibacterial activity of methanolic, ethanolic, ethyl acetate and aqueous extract of stem and leaves of *Tridax procumbens* and roots *of A. mexicana* were tested for their antibacterial potential against *Salmonella typhi, Bacillus substilis* and *Staphylococcus aureus* and *Aeromonas.* The ethylacetate extracts of the *Tridax procumbens* showed effective inhibition against all four bacterial species.Comparatively, the ethyl acetate extract of *T. procumbens* and *A. Mexicana* showed higher antibacterial activity against all four bacterial species than other extract while *Staphylococcus aureus* showed more sensitivity to ethyl acetate extract of *T. procumbens* and *A. mexicana.*

KEYWORDS: Antibacterial, *Argemone mexicana, Tridax procumbens, Salmonella typhi, Bacillus subtilis.*

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1. INTRODUCTION

One of the leading causes of the death throughout the world is infectious diseases and the resistance has grown within the infectious organisms against the antibiotics. In addition, antibiotics used sometime shows adverse effects including hypersensitivity reactions, immune suppression and allergic reactions. To combat this problem, there is a continuous search of new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases [4]. The natural compounds obtained from plants can provide potential mean for the development of new drug [3]. Nowadays researchers are screening various plants regarding this issue [6-8]. In different parts of the world, many plants and their parts are used for the treatment of various diseases and are being screened for their antimicrobial activities [9]. Recently, researchers are also investigating to find out antimicrobial synergism within different plant extracts [10].In this study we used two plants (*Argemone mexicana and Tridax procumbens*) individually and in combination to test their synergistic antibacterial potential. Both of these plants known to have medicinal properties. *T. procumbens* (L) is a common medicinal herb belonging to family Asteraceae and is used by ethno-medical practitioners as antifungal, antimicrobial agents.The tribal peoples in some Indian states uses leaf juice to cure fresh wounds, as a hair tonic and to stop bleeding [11-13].The another plant used for antibacterial study is prickly poppy plant, *Argemone Mexicana* traditionally used as healing agent in treatments of skin diseases, malarial infection, warts, cold sores, itches [12]. Scientifically, leaf-extracts of A. mexicana were used to examine a antibacterial potentiality against several pathogenic bacteria [14]. The present study has been undertaken to test the antibacterial potential of *T. procumbens* and *A. mexicana* individually and synergistically.

2. MATERIALS AND METHODS

Plant Material used

The plant, *A. mexicana* and *T. procumbens* were collected from Shrigonda Tehsil. The taxonomic identifications of these plants were carried out in Botany department of M.J.S. College Shrigonda. After identification of plants, the roots *of A. mexicana* and stem and leaves of *T. procumbens* were cleaned under running tap water, chopped into small pieces and air dried under shade for ten days. After proper drying the plant material were pulverized into powdered form by grinder.

Preparation of Plant Extract

The air dried powder material (5 gm each) of both plants was extracted with 100 ml of water, ethanol, methanol and ethyl acetate separately by soxhlet apparatus. The extract was concentrated by evaporating the solvent by rotary evaporator. The concentrated extract was air dried and diluted to 4 mg/ml of each solvent respectively and used for further study. For synergistic activity, 1:1 mixture of *A. mexicana* and *T. procumbens* of each solvent from diluted stock were taken and mixed together and further used for testing of synergistic antibacterial activity.

Microorganism used

For this study, *Salmonella typhi, Bacillus subtilis, Staphylococcus aureus* and *Aeromonas* were used as test organisms. Bacteria used for the study were obtained from Department of Microbiology, University of Pune. All the cultures were maintained at 4˚C in nutrient agar slants.

Antibacterial activity

The antibacterial activity of *T. procumbens* and *A. mexicana* and combined extract of both plants were tested by agar well diffusion assay [15]. For the agar well diffusion method, nutrient agar plates were seeded with standard inoculum (1×10^6 CFU/ml) of the test microorganism. Wells of 10mm diameter were prepared in the plates with a sterile borer and 50 microlitres of the sample extract of each plant was added directly into the wells. For synergistic antibacterial activity testing, 1:1 mixture of both plants extract was taken and 50 microliters of extract was pipette out into the wells. The plates were incubated overnight at 37°C. Antibacterial activity was determined by measuring the diameter of the zones of inhibition of bacterial growth surrounding the wells. The effective zone size of the each extract was determined by subtracting the zone size of the solvent control of the respective extract.

3. RESULTS AND DISCUSSION

Table1: Antibacterial activity of *Tridax procumbens* and *Argemone mexicana.*

T.p- *Tridax procumbens*, A.m- *Argemone mexicana*

Bacterial Strain	Inhibition zone (mm)							
	Methanol		Ethanol		Ethyl acetate		Aqueous	
	T.p	A.m	T.p	A.m	T.p	A.m	T.p	A.m
S. typhi	No zone (R	2.5	3.5	4.0	6.8	8.5	No zone (R)	3.0
B. subtilis	6	7.5	5	6.5	7.5	8.0	No zone (R)	4.0
S. aureus	7.5	5.0	5.0	5.5	8.0	10	4.0	6.0
Aeromonas	6.5	4.5	2.5	3	7.5	9.5	No zone (R)	4.5

Table2: Synergistic activity of *T. procumbens* and *A. mexicana* plant extract in combination (1:1 mixture).

Bacterial strain	Combined extract, inhibition zone (mm)			
	Methanol	Ethanol	Ethyl acetate	Aqueous
S. typhi	7.4	5.5	9.0	4.0
B. substilis	8.0	7.5	8.2	4.2
S. aureus	8.2	6.8	11.2	7.0
Aeromonas	6.8	4.2	10.4	4.4

In India, 70% of its population resides in villages. In spite of the accessibility to western medicine, people in these villages still continue to depend on herbal remedies, for treatment of their health problems. Plant species have long been the principal ingredients of traditional medicine and their use dates back to the beginning of human civilization. As compared to synthetic antimicrobial agents, plant based antimicrobials are cost effective, affordable and exhibit lesser side effects [16].The present aim of our study was to find out the antibacterial potential of *A. mexicana* and *T. procumbens* and also the synergistic activity of combined extract of these two plants. The roots of A. mexicana and stem and leaves of *T. procumbens were u*sed to prepare methanolic, ethanolic, ethyl acetate and aqueous extract. The concentrated extract of each plant and in combination also were inoculated into wells prepared in pre-seeded agar plates, as described for the agar well diffusion assay [15]. After incubation at 37°C for 24 h, the zone of inhibition around the well was measured and recorded as the measure of antibacterial activity. Results depicted in table.1 shows that the Ethyl acetate extract of *A. mexicana* root exhibit highest significant antibacterial activity against all four bacterial strains. Whereas the *S. typhi, B. subtilis and Aeromonas shows* resistance against aqueous extract of *T. procumbence.* The synergistic activity of *T. procumbens* and *A. mexicana* plant extract were also studied. In Synergistic study it is observed that upon combination of these two plants extract, the aqueous extract also shows the significant antibacterial activity against all four bacterial strains. It is also shown in table. 2 that the mixed ethyl acetate extract of both these plants, elicitates the antibacterial potential when compared with individual extract. These observations suggest that the A. mexicana root extract and T. procumbens leaf and stem extract contains an antibacterial potentiality and this potentiality increases when acted synergistically. Chopra et al., 1986; Siddiqui et al., 2002; Osho & Adentunji 2010; Rubio-Piña & Vázquez- Flota, 2013, also demonstrated that oil extracts of *A. mexicana*, at various levels of concentration, inhibit growth of filamentous fungi and non- filamentous fungus *C. albicans*, along with a few bacteria, such as *Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa. The* evaluation of the antibacterial activity of crude extracts of root, stem, leaves and seed of *A. mexicana* against Gram positive bacteria *(B. subtilis, B. cerus, S. aureus, Streptococcus agalactae, Enterobacter faecalie, C. botulinum, C. perfringens and* Gram negative bacteria *E. coli*, *S. typhi, P. mirabilis, P. vulgaris, K. pneumoniae, P. aeruginosa.*

4. CONCLUSION

Thus, from present study of investigation it can be concluded that the higher antimicrobial potential is shown by synergistic activity of A. mexicana and T. procumbens when compared with individual plant extract.

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CONFLICT OF INTEREST

There is no conflict of interest.

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