ISSN: 2320 - 4230

Journal of Drug Discovery and Therapeutics 1 (5) 2013, 17-20

RESEARCH ARTICLE

EVALUATION OF SEED OIL OF *ZANTHOXYLUM ARMATUM*FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Essential oils have proved to possess valuable antimicrobial and antimicrobial activity. The present work aims at finding out potential of essential oils whose antimicrobial activities have not been fully explored. The review of literature suggests that the essential oil of Zanthoxylum has good antimicrobial potential. It was considered to procure essential oil from the market and evaluate the same for chemical consistency and antimicrobial activity. This strengthens the belief that we should also undertake studies which should be of commercial significance and not only screening of medicinal plants. It is therefore, imperative to search alternative drugs for the treatment of microbial diseases to replace the existing drugs of doubtful efficacy and safety. The present research paper reveals the evaluation of seed oil of *Zanthoxylum armatum* for Antimicrobial Activity.

KEY WORDS: *Zanthoxylum armatum,* Essential oil, Antimicrobial Activity.

INTRODUCTION:

Plants are widely used in ethno-medicine around the world. Historically, therapeutic results have been mixed; quite often cures or symptomatic relief resulted. Poisonings occurred at a high rate, also. Currently, one-quarter to one-half of all pharmaceuticals dispensed in the United States having higher-plant origins, very few are intended to use as antimicrobials, since we have relied on bacterial and fungal sources for these activities[1]. The antimicrobial activities of many plant-derived compounds are well known and in particular, the antimicrobial activities of essential oils and their constituents, which are mainly terpenes, have been studied in some details[2]. The antimicrobial activities of aromatic substances have been known for more than seventy years. Among the natural products responsible for antimicrobial activities are isolated constituents such as flavones,[3] xanthones, anthranilate derivatives, anthraquinone derivatives (rhein, physcion, aloe- emodin and chrysophenol), diterpenoid alkaloids (vilmprianone, panicutine, 8-acetyl-heterophylhisine), volatile oils etc.[4,5,6]

ZANTHOXYLUM ARMATUM DC (TIMUR):

Z. armatum belongs to *Rutaceae* and several species possess medicinal values like anaesthetic, antibacterial, antifungal, anti-inflammatory, oro-dental care etc. Fruits of *Z. armatum* are known to contain phytoconstituents like resins, alkaloids, and volatile oils. Zanthoxylum is high in a Linalool. Linalool high oils offer us profound healing for infections, pain, swelling, spasms, allergies and injury. The oil is also deeply calming for the nervous system. It can be used daily and is a wonderful tonic for health[7].

Zanthoxylum armatum DC (Rutaceae) is extensively used in the Indian system of medicine, as carminative, stomachic and anthelmintic. The bark is pungent and stick from the plant is used in preventing toothache. The fruits and seeds are employed as an aromatic tonic in fever, dyspepsia, and expelling roundworms[8]. Mehta *et al* has reported that the essential oil of fruits of *Z. armatum* exhibited good antibacterial, antifungal and anthelmintic activities[11]. Kokate *et al* reported that the petroleum ether extract of *Z. armatum*, showed significant insecticidal activity against *Culex* sp. with LC50 value of 20.45 ppm[9].

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Figure 1: Plant and Seeds of *Zanthoxylum armatum DC (Timur*)

MATERIAL AND METHOD:

MATERIAL:

The following chemicals, glassware, equipments and drug samples were used:

Table 1: Chemicals used

Sr. No	Chemicals	Specification	Manufacturer / Supplier
1.	DPPH	AR	RFCL Ltd. New Delhi
2.	Dichloromethane	AR	Merck specialities pvt Ltd. Mumbai
3.	Ethanol	AR	RFCL Ltd. New Delhi
4.	Helium gas	AR	Qualigens Fine chemical Mumbai.
5.	Methanol	AR	RFCL Ltd. New Delhi
6.	N-Hexane	AR	Merck specialities pvt Ltd. Mumbai
7.	Petroleum ether	AR	RFCL Ltd. New Delhi
8.	Phenol peptone	AR	Merck specialities pvt Ltd. Mumbai
9.	Sodium Chloride	AR	Merck specialities pvt Ltd. Mumbai

Table 2: Glassware used

Sr. No.	Glassware	Specification / Size	Manufacturer / Supplier
1.	Beakers	100 mL, 250 mL	Borosil Glass Works Ltd. Worli, Mumbai
2.	Conical flasks	250 mL	Borosil Glass Works Ltd. Worli, Mumbai
3.	Funnel	50 mm	Borosil Glass Works Ltd. Worli, Mumbai
4.	Glass plates	15	Ordinary
5.	Measuring cylinder	50 mL, 100 mL	Borosil Glass Works Ltd. Worli, Mumbai
6.	Pipettes	1 mL, 5 mL, 10 mL	Borosil Glass Works Ltd. Worli, Mumbai
7.	Spiral glass condenser	500 ml	Borosil Glass Works Ltd. Worli, Mumbai
8.	Volumetric flask	100 mL, 250 mL	Borosil Glass Works Ltd. Worli, Mumbai
9.	Weighing bottle	50 mL	Borosil Glass Works Ltd. Worli, Mumbai

Table 3: Apparatus / Equipments used

Sr. No.	Apparatus / equipments	Model	Manufacturer / Supplier
1.	Digital Balance	ST 123	Citron Electronic
2.	Camera	Cyber shot 7x DSQ 35	Sony
3.	Muffle furnace	SES 6649	Swastika Elec. & Sc. Works

Table 4: Drugs used

Sr. No.	Drugs	Company
1.	Gentamicin sulphate	Nicolas Piramal
2.	Kanamycin	Macleods Pharmaceutical

METHOD:

♦ **COLLECTION OF THE PLANT MATERIAL:**

The plant material *Z. armatum* was collected from Akhori, District-Chamoli, Uttarakhand. The plant material was authenticated at Department of Pharmacy, Kumaun University, Bhimtal and a voucher sample was submitted there.

♦ **EXTRACTION OF THE ESSENTIAL OIL:**

The fresh plant material 1.0 kg was subjected to steam distillation using a copper electric still, fitted with a spiral glass condenser. The distillate was saturated with NaCl and the oil was extracted with n- hexane and dichloromethane. The organic phase was dried over anhydrous sodium sulphate and the solvent was distilled in rotary vacuum evaporator at 35^{0}C. Yield was 0.62% w/w.

♦ **EVALUATION FOR ANTIMICROBIAL ACTIVITY:**

Antimicrobial assays were done by serial dilution as per the method described.[10, 11]

BACTERIAL AND FUNGAL CULTURES:

All the microorganisms used were isolated pathogens. The pathogens were procured from the Siddhartha Institute of Pharmacy, Dehradun. Cultures were maintained in the laboratory by regular sub culturing media.

STRAINS AND STANDARDS:

The bacterial strains selected for study were *E. coli, S. aureus, Bacillus cereus* (Var. mycoides). Fungal strains selected for study were *Candida albicans Aspergillus niger*, *Cryptococcyus neoformans*.

PREPARATION OF THE MEDIA:

The experiment was performed using broth culture method. Basic medium was prepared using the following formula:

Proteose peptone	: 10 gm
NaCl	: 5gm
Phenol Red Solution	: 4.5ml
Distilled Water q.s.	: 1000ml

Media was prepared by dissolving all the ingredients in distilled water. The media was sterilized by autoclaving at 121^{o}C for 15 minutes.

The standard drugs used were Gentamicin sulphate and Kanamycin.

PREPARATION OF CULTURES BY SERIAL DILUTION:

For the preparation of culture, nutrient broth was taken in a test tube and a loop full of bacterial/ fungal samples was added with the help of inoculating loop.

ISOLATION OF COLONY:

Six test tubes were taken and marked as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}. The tubes were filled with 9 ml of nutrient media. Tube one was inoculated with 1ml of culture. All the test tubes were inoculated from previous tubes subsequently. Serial dilution helps in easy isolation of single colony of bacteria. The serially diluted tube marked 10^{-5} was used for the final evaluation. The results will be calculated by statistical method.

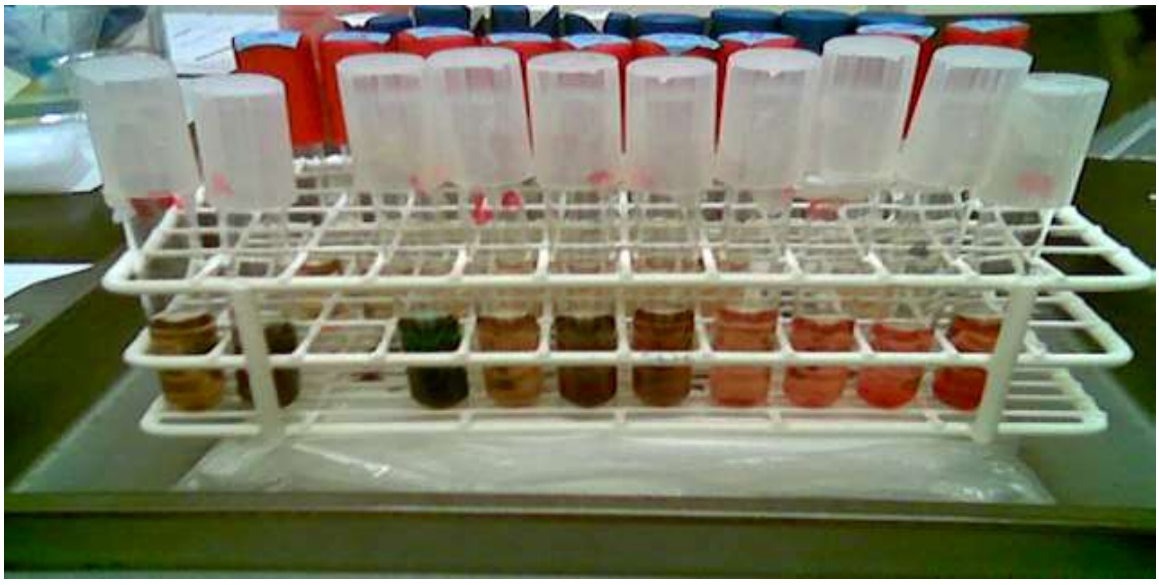

Figure 2: Zone of inhibition; antimicrobial activity of *Z. armatum* DC by serial dilution method.

RESULT AND DISSCUSSION:

The antimicrobial activities of many plant-derived compounds are well known and in particular, the antimicrobial activities of essential oils and their constituents, which are mainly terpens, have been studied in some details.

From the above experiment and method, the following result was found regarding the activity of the seed oil of *Z. armatum* against test microbes:

Table 5: Antimicrobial activity of seed oil of *Z. armatum*

Sr. No.	Strains	% Activity (Zone of Inhibition)
1.	*E. coli*	75.4%
2.	*S. aureus*	71.2%
3.	*Bacillus cereus* var. mycoides	98.4%
4.	*Candida albicans*	97.9%
5.	*Aspergillus niger*	67.8%
6.	*Cryptococcyus neoformans*	85.0%

From the study it was found that the oil was highly effective against *Bacillus cereus var. mycoides, Candida albicans and Cryptococcus neoformans.* The oil showed good activity against *S. aureus, E. coli* and *A. niger*.

CONCLUSION:

It has been reported that the *Zanthoxylum armatum DC* essential oil possess strong antimicrobial agent. The oil was found especially effective against *E. coli, Bacillus cereus var. mycoides, Candida albicans, Cryptococcus neoformans.* Thus from the study and results it is concluded that plant *Z. armatum* has a very good scope in developing herbal antimicrobials with varied degree of activities.

REFERENCES:

1. William E.C., Trease and Evans, Pharmacognosy, Fifteenth edition, Elsevier Ltd China, 2002, 134-135.
2. Berry JP, Mecferren MA, Rodriguez E, Zoopharmacognosy: A biorational strategy for phytochemical prospecting. In: Gustine DL, Flores H. (eds.) Phytochemicals and health, ASPP, Rockwville, 1995; 165-178.
3. http://nmpb.nic.in (National Medicinal Plants Board, GoI website).
4. Mukherjee PK, Dixit VK. Quality control of herbal drugs. First edition Business horizons.2002; 427.
5. Botham P.A., Acute systemic toxicity. ILAR Journal. 2002(43): S27-S29.
6. Stockwell**,** C. **(**1988). Nature's Pharmacy. Century Hutchinson Ltd., London, United Kingdom.
7. Sun, H. D., S. X. Qiu, L. Z. Lin, Z. Y. Wang, Z. W. Lin, T. Pengsuparp, J. M. Pezzuto, H. H. Fong, G. A. Cordell, and N. R.Farnsworth.(1996). Nigranoic acid, a triterpenoid from *Schisandra sphaerandra* that inhibits HIV-1 reverse transcriptase. *J. Nat. Prod*. 59:525–527.
8. Pezzuto, H. H. Fong, G. A. Cordell, and N. R. Farnsworth **(**1996). Nigranoic acid, a triterpenoid from *Schisandra sphaerandra* that inhibits HIV-1 reverse transcriptase. *J. Nat. Prod*. 59:525–527.
9. Cortez, D.A.G., Young, M.C.M., Martson, A., Wolfender, J.L. and Hostettmann, K. (1998). *Phytochemistry*, 47(7), 1367.
10. Indian Pharmacopoeia, 2007.Vol I, Indian Pharmacopoeia Commission, 2008, p.2368.
11. Pelczar MJ, Chan ECS, Krieg NR (1993). Microbilogy: Concepts and Applications, McGraw and Hill, Newyork. p.578-579.