IN VITRO ANTIBACTERIAL POTENTIAL OF *AEGLE MARMELOS* (L.) CALLUS EXTRACT

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Summary

Antibacterial potential of crude petroleum ether extract obtained from leaf callus tissue of *Aegle marmelos* was evaluated against five bacterial pathogens using agar well diffusion method. Among the tested bacterial strains, inhibitory activity of the callus extract was minimum against *Klebsiella pneumoniae*(4mm) and it was followed by *Proteus vulgaris* (5mm). Maximum inhibitory activity was observed against *Salmonella typhi* (12mm). From this observation it is evident that phytochemical principles which are responsible for the curative (antibacterial) activity of *Aegle marmelos* could have a constant expression pattern in a specialized set of cells even after their rapid division and differentiation into callus form. *In vitro* micropropagated clones from the parental plant will have an efficient transmission of the antibacterial active constituents. Moreover, this observation may be a baseline for the large scale extraction of antibacterial principles and other curative chemicals through callus culture.

Key Words: *Aegle marmelos*, Callus culture, antibacterial principles, herbal medicine

Introduction

Finding healing powers with plants is an ancient idea. People on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory. There is evidence that Neanderthals living 60,000 years ago in present day Iraq used plants such as hollyhock. These plants are still widely used in ethnomedicine around the world. Historically, therapeutic results have been mixed; quite often cures or symptom relief resulted. Poisonings occurred at a high rate, also. Currently, of the one-quarter to one-half of all pharmaceuticals dispensed in the United States having higher plant origins, very few are intended for use as antimicrobials, since we have relied on bacterial and fungal sources for these activities. Since the advent of antibiotics in the 1950s, the use of plant derivatives as antimicrobials has been visually nonexistent (6).

In India, herbal medicines have been the basis of treatment and cure for various diseases, physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Indian folk medicine comprises numerous herbal prescriptions for therapeutic purposes which may be as varied as healing wounds, treating inflammation due to infection, skin lesions, leprosy, diarrhoea, scabies, venereal diseases, snake bite and ulcers etc. The realization that many infectious pathogenic microorganisms are fast developing resistance against prevailing drugs has necessitated a search for new sources of anti-microbial compounds. In the course of their life cycle, plants encounter infection by a variety of viruses, bacteria, fungi, parasites specific to them. They are expected to synthesize a variety of secondary metabolites capable of providing them protection against the infectious agents (5).

Investigations into the Antimicrobial activities, mode of action and potential uses of plant valatile oils have regained momentum. There appears to be a revival in the use of traditional approaches to protecting livestock and food from disease, pests and spoilage in industrial countries. This is especially true in regard to plant volatile oils and their

Antimicrobial evaluation, as can be seen from comprehensive range of organisms been tested. The Antimicrobial properties of plant volatile oils and their constituents from a wide variety of plants have been assessed (1). These have included food spoiling organisms and food poisoning organisms spoilage and mycotoxigenic filamentous fungi and plant viruses.

Although reports of antibacterial activity of indigenous plants have been known from many regions (2) they have not been systematically and adequately evaluated and validated, except in a few cases, thereby leading to confusion in drawing a meaningful conclusions. Hence, in every developing country it is necessary that the documentation of medicinal plants be treated as a matter of extreme urgency (13).

About *Aegle marmelos*

It belongs to the family Rutaceae and is being cultivated and planted as a temple tree (Shthala Virutcham) especially in the temples of Lord Shiva. It is otherwise called as “wood apple” or “Beal”. It is a deciduous tree grow up to the height of 30-40 feet in Indian peninsular, dry hilly places of Western Himalaya to altitude of 4000 feet. Usually being propagated by seeds. Almost all parts of this plant are medicinal and economic important ones. Marmelosin is an active constituent which is partially responsible for such medicinal value of this plant species(4) carried out preliminary chemical examination of root, bark, leaves, fruit and seeds. Pharmacognosy of root bark, stem bark had been studied in detail by Prakash and Prasad (9). Though this plant is being treated as a highly medicinal one there is no detailed information about its utility for the specific ailments. This lack of information is due to the absence of a thorough analysis of the active constituents of this plant against selective group of pathogenic bacteria which are responsible for such diseases. Moreover, there is a thrust over the phytochemists regarding the detainment of medicinal properties of the mother plants into their clones when they are being subjected to *in vitro* regeneration practices.

In this present study, such a preliminary attempt was made to evaluate the active phytochemical constituents present in the callus tissues obtained from the leaf disc of

Aegle marmelos. Actively proliferating callus tissue was obtained from leaf disc explant of *Aegle marmelos* by culturing the young leaf discs in sterile MS medium supplemented with 2 micromolar Kinetin and 6 micromolar 2,4-D. Callus tissue was established in the same media combination. In this Crude petroleum ether extract was obtained from that callus and its antibacterial potential in vitro was evaluated against five selected bacterial pathogens using agar well diffusion method.

Materials and Methods

8mm fresh leaf discs (obtained from tender leaves) were inoculated after surface sterilization in sterile Murashige and Skoog (1962) medium supplemented with different concentrations of Kinetin and 2,4-D in combination. The best media combination was identified based on the callus formation efficiency and further cultures was done with such media combination alone for establishing the callus for further extraction. Established callus tissue (both friable and non friable) was used for the crude extract preparation. 1 gram of callus tissue was ground finely with 20ml of petroleum ether as solvent. Then the extract was filtered aseptically using several layers of sterile cheese cloth. 50 and 100 microlitre of this filtrate (separately) was loaded in 6mm agar wells created in sterile nutrient agar Petri dishes spread with over night grown pathogenic bacterial cultures separately. In each set triplicate Petri dishes were maintained. For negative control one well was loaded with 100 microlitre of petroleum ether and for positive control, 100 microlitre of gentamycin antibiotic solution (50mg/ml) was also loaded. Inoculated Petri dishes were incubated at 37ºC for 20- 24 hours. After the incubation, zone of inhibition around the wells were observed and recorded, average values were calculated and tabulated (Table.1).

Results

1g/20ml concentration was effective against all the bacterial strains used in this present study. In case of the extracts used in different volumes, 100 microlitre was yielded with notable and interesting results i.e. a clear zone of inhibition. It could be the minimal volume for the exhibition of the antibacterial potential of callus extract. Based on this

present observation, the minimal inhibitory concentration of the callus crude extract could be 5mg/ml. It reveals that the existing presence of the antibacterial principles need to be more than the mother plant tissues comparatively.

Table.1. Inhibitory activity of *Aegle marmelos* leaf callus extract (Test), Petroleum ether (- control) and Gentamycin antibiotic (+ control) against the selected bacterial strains (Zone of inhibition in mm - diameter of agar well ± SD. All values are average of triplicates)

S.No.	*Salmonella typhi*	*Proteus vulgaris*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Escherichia coli*
Test *(Aegle marmelos* callus extract)	**12 ± 0.202**	**5 ± 0.143**	**4 ± 0.135**	**8 ± 0.164**	**11 ± 0.198**
Negative control	**2 ± 0.197**	**3 ± 0.204**	**3 ± 0.208**	**2 ± 0.186**	**2 ± 0.208**
Positive control	**9 ± 0.214**	**8 ± 0.210**	**8 ± 0.209**	**9 ± 0.182**	**8 ± 0.191**

Discussion

Among the listed medicinal and aromatic plants in medicinal flora, *Aegle marmelos* and its medicinal properties were listed within "Charuka samhita" an early medical treatise. This categorization will give a special attention to this tree species other than its significance as a sacred groove tree. Juice of *A. marmelos* leaves proved to be a better medicine for hyper – excitability and running nose, memory power improvement, dysentery and intermittent fevers (12, 7). Callus extract exhibited more inhibitory effects against *S. typhi* and *E. coli.* These findings are supporting the previous reports by Rastogi (1993) and Majumdar (2002). In case of *K. peumoniae* and *P.vulgaris*, such a high efficiency of inhibition was not observed.

This may be due to the development of multiple drug resistance by the respective bacterial strains during the course of their differential exposure. This modified pattern of

inhibition reveals that this plant based medicine could be fully screened and the possible number of bacterial infections could be controlled by the application of *Aegle* plant parts. So, that the broad spectrum as well as the blunt usage of herbal medicines for a non prescribed ailments will get regulated. Moreover, the possible side effects due to such blunt usage of herbal medicines may be gradually reduced.

Even in this less concentration i.e 5mg/ml of MIC this callus extract was able to exhibit such a promising inhibition against almost all the tested pathogenic bacteria. This reveals that the specialized group of this plant cells are capable of synthesizing and storing such antibacterial active constituents in higher quantity. So, further studies regarding the specific tissues or cells involved in this synthesis/secretory phenomenon. If it so happens, the selection of the suitable explant and exploitation of such explant for the in vitro production of antibacterials could become an easy and more convenient ones. From this present study it is evident that the antibacterial active constituent of this plant is having a constant expression pattern and it is being detained into the derived callus. So, no doubt the similar medicinal properties of this parental plant will be transmitted fully to clonal plantlets could be produced through the in vitro micropropagation protocol. So, in vitro cultivation and extraction of active chemical constituents may be a promising ones without the loss of their significance. In order to evaluate the constant transmission of antibacterial principles to the next progenies, further studies are underway to testify the antibacterial potential of the plantlets which are being produced from the callus through in vitro regeneration.

Further studies need to be undertaken to analyze the correlation between the chemical composition of the leaf based antibacterial substances and their specific activity against the particular group of bacterial pathogens as reported by Cimanga *et al*., (2002).

Acknowledgement

Authors are thankful to Dr.P. Ravichandran, Reader, SPKCES, M.S.University, Alwarkurichy, for valuable suggestions. Authors are also thankful to the Principal, Secretary and Management of Sri Pramakalyani College, Alwarkurichi for their encouragement and support.

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