



## Antibacterial Activity of Regenerates of *Majorana Hortensis Moench*

### KEYWORDS

*Majorana hortensis*, Regenerates, Methanol extract, Antibacterial activity.

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**ABSTRACT** *The antibacterial activity of the methanol and petroleum ether extracts of the normal and regenerated plants of Majorana hortensis Moench was tested against six bacteria (Serratia marcescens, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, E. coli-XL Blue and Klebsiella pneumoniae) by well diffusion method. The study showed that all six organisms were sensitive to the methanol extract of the plant sample. Further, the extract of the regenerated plant was superior than that of the normal plant in its antibacterial activity. Significant activity was observed against E.coli, Serratia marcescens and Proteus vulgaris, moderate activity against Staphylococcus aureus while least effect was observed against Pseudomonas aeruginosa and Klebsiella pneumoniae.*

### INTRODUCTION

The use of herbs in the treatment of various human ailments has been known to mankind since times immemorial. The medicinal value of the drug plant is due to the presence of some chemical substances<sup>1</sup>. These substances can be used for therapeutic purpose or they are the precursors for the synthesis of new drugs<sup>2</sup>. A number of plants have used in traditional medicine for many years due to their antimicrobial properties<sup>3</sup>. (Sofowora, 1993). Specifically the medicinal value of these plants lies in some chemical substances that produce definite physiological action on the human or animal body<sup>4</sup>. The active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive<sup>5</sup>. However these complementary components give the plant as a whole, a safety and efficiency much superior to that of its isolated and pure active components<sup>6</sup>. The most important of these bioactive constituents which are mainly secondary metabolites are alkaloids, flavonoids, tannins, terpenoids, phenolic compounds and essential oils which have antimicrobial properties<sup>7, 8</sup>. These phytochemicals are toxic to microbial cells<sup>2</sup>. Essential oils have been amply documented to kill a wide range of pathogenic fungi and bacteria, such as *Candida albicans*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* including their drug resistant variants<sup>9,10,11,12</sup>. The incidence of resistance in human pathogenic microorganisms in recent years perhaps is due to indiscriminate use of commercial antimicrobial drugs. Gram negative bacteria possess increasing resistance to antibiotics<sup>13,14</sup>. Further, antibiotics are sometimes associated with side effects and toxicity<sup>15,16</sup>. This has forced the scientists to look for new antimicrobial substances from various sources including the medicinal plants<sup>17</sup>. Recent works have revealed the potential of several herbs as sources of drugs<sup>18</sup>. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes<sup>19</sup>. Many natural antimicrobial compounds can be derived from plants<sup>20</sup>. Ahmed et al.,<sup>16</sup> opined that medicinal plants represent a rich source from which new antibacterial and antifungal chemotherapeutic agents may be obtained. Numerous studies have identified compounds within herbal plants that are effective antibiotics<sup>21</sup>. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials<sup>22,23</sup>. Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics<sup>24</sup>. Some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria<sup>25</sup>. The results indicate the need for further research into traditional health systems<sup>26</sup>. It also

facilitates pharmacological studies leading to the synthesis of more potent drugs with reduced toxicity<sup>27, 28</sup>. In recent years several workers<sup>29,30,31,32</sup> have screened many plants for their antibacterial properties.

The genus *Majorana hortensis Moench* belonging to the family Lamiaceae is an important aromatic medicinal herb widely used Ayurveda and Unani systems of medicine to cure various human ailments. The volatile oil commercially known as the Oil of sweet Marjoram is obtained by the steam distillation of leaves and flower heads of the plant. Perusal of literature has revealed its high medicinal value. Marjoram was initially used by Hippocrates as an antiseptic agent<sup>33</sup>. It is a well-liked home remedy for chest infection, cough, sore throat, rheumatic pain, nervous disorders, cardiovascular diseases, epilepsy, insomnia, skin care, leucoderma, inflammation<sup>34</sup>, flatulence and stomach disorders<sup>35</sup>. An infusion of the plant is used as stimulant, sudorific, emmenagogue and useful against Asthma, Hysteria and paralysis<sup>36</sup>. The antiproliferative activity of Marjoram was confirmed in human lymphoblastic leukemia cell line Jurkat<sup>37</sup>. Marjoram species have been reported to show significant antimicrobial activity and its prolonged use may reduce gut bacteria<sup>38, 39, 40</sup>. The plant is reported to possess antibacterial properties<sup>41, 36</sup>. In the present investigation, an attempt is made to study the antibacterial effect of the crude extract of the leaves of regenerates of *Majorana hortensis* raised on media supplemented with growth regulators.

### MATERIALS AND METHODS:

#### Plant material

The aerial parts of the normal plants of *Majorana hortensis* which were maintained in the garden of the Dept. of Botany, Bangalore University were collected, shade dried and powdered and used as sample 1 for extraction.

The aerial parts of micropropagated plants raised on culture media fortified with growth regulators, maintained in the garden of the Dept. of Botany, Bangalore University were collected, shade dried, powdered and used as sample 2 for extraction.

#### Preparation of the crude extract:

The antimicrobial activity was examined for the methanol and petroleum ether extracts of sample 1 and 2. Methanol and petroleum ether were used for the preparation of the crude extract.

0.5 g. of each of the samples 1 and 2 was extracted in 7 ml. of the solvents. The contents were transferred to a bottle and

stirred at room temp and extraction was allowed to proceed for 48 hours. After extraction the samples were centrifuged at 10,000 rpm for 10 min. The supernatant is concentrated by evaporation. The concentrated solid extracts were reconstituted by dissolving in 0.2 ml of the solvent and stored at 4°C for further antibacterial studies.

#### The plant extracts were tested against the following pathogenic Bacteria :

1. *Serratia marcescens*: a gram -ve rod. Causes urinary tract infections, hospital epidemics, septicaemia, peritonitis, arthritis, pneumonia.
2. *Proteus vulgaris*: a gram -ve, enterobacterium, causes urinary tract infection.
3. *Staphylococcus aureus* : a gram +ve cocci in clusters that causes skin infections, skin lesions, abscesses, boils, scalded skin.
4. *E.coli- XL Blue*: a gram -ve rod shaped bacteria living as a parasite in the human and animal intestine; may cause enteric diseases, haemorrhage colitis and urinary tract infection.
5. *Pseudomonas aeruginosa*: a gram -ve aerobic bacillus living in the soil, water, sewage, mammalian gut and plants. It causes nosocomial infections including metabolic, haematological and malignant diseases. It is also known to cause severe epidemic diarrhoea in infants, ocular infections, osteomyelitis etc.
6. *Klebsiella pneumoniae*: a gram -ve ,enterobacterium, causes urinary tract infections, broncho and lobar pneumonia where in the entire lobe of the lung is infected.

The strains were obtained from Bangalore Genie where they are maintained as pure cultures.

#### The growth media used for bacterial cultures:

Mueller Hinton Agar (MHA from Hi media) was used for *S.marcenscens*, *P.vulgaris*, *S. aureus*, *E. coli XL Blue* and *K. pneumoniae*. Luria Bertani Agar (LB Agar) for *P. aeruginosa*.

#### Preparation of MHA medium:

38 g. of MHA (Hi media) was dissolved in 1 litre of distilled water.

#### Preparation of Luria Bertani Agar medium:

Tryptone -----10 g

Yeast extract--5 g

NaCl-----10 g

Dissolved in 500 ml. of water. The pH was adjusted to 7 using 1N NaOH

Agar-----17.5 g

Both MHA and LBA media were autoclaved at 121°C for 15 min and stored at RT for further use.

#### Antibiotics used:

Cephalothin (Ce) against *Serratia*, *Proteus*, *E.coli* and *Klebsiella*

Ampicillin (A) against *Staphylococcus*

Spectinomycin (Sp) against *Pseudomonas*

The glassware and other instruments needed for inoculation were sterilized in the hot air oven at 160°C for 1 hr. The process of inoculation was carried out in an aseptic environment in a laminar airflow chamber which is previously clean swabbed with 90% alcohol.

#### Antibacterial assay:

Antibacterial assay was carried out by Well diffusion method<sup>42</sup>.

1 ml. of the inoculum was centrifuged at 6000 rpm for 10 min. The pellet was suspended in 0.3 ml of LB Broth (LBA without agar). 100 µl of the suspended inoculum was spread on MHA and LBA media using a sterilized spreader to get a uniform lawn of the bacteria. The surface of the agar was allowed to dry before punching wells and placing the antibiotic discs. In each plate two wells of 0.5 mm. each were punched using a gel puncher and were marked on the reverse. 20 µl each of the reconstituted extract and the respective solvent (negative control) were added to the respective wells and an antibiotic disc was placed as a +ve control. Thus each plate contained one well with the sample extract, one well with the solvent control and an appropriate antibiotic disc.

The plates were incubated at 37°C for 24 hrs. The zone of inhibition induced by the extract and the controls on the growth of microorganisms were measured in mm. Each experiment was triplicated and the average values were tabulated.

#### Results and Discussion:

The antibacterial activities of the extracts of normal and regenerated plants were tested against six pathogenic bacteria and their effect and potency were assessed by measuring the diameter of the zone of inhibition. The inhibition zones due to the effect of plant extracts were compared with those due to the respective solvents (-ve control) and the antibiotics (+ve control). The inhibitory effect of solvent alone is nil in all the tests conducted and therefore is not included in the table while the effect of the various antibiotics is recorded in all the cases.

The results revealed that the methanol extracts of both the normal and regenerated plant samples was effective against all six pathogenic bacteria. Similar antibacterial activity of methanol extracts was observed by Leeja and Thoppil<sup>40</sup> in the same taxon. But the petroleum ether extracts did not show any inhibitory effect. Isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure<sup>5</sup>. The absence of inhibition zone in the petroleum ether extract could perhaps be due to the non-solubility of active constituents in petroleum ether while the methanol extract may have more of the bio-active compounds.

Further the extract of the regenerated plant showed greater inhibitory zones against the pathogens than the extract of the normal plant. This is in conformity with the observations of Sowmya et al.,<sup>43</sup> in *Bacopa monnieri*.

It was observed that the extract of the normal plant is most effective against *E.coli* (12.0±0.15) and moderate against *S.aureus* (10.0±0.15) and *S.marcescens* (7.96±0.15) while it is least against *P.vulgaris* (7.0±0.10) *P.aeruginosa* (7.0±0.10) and *K.pneumoniae*(6.93±0.05). In comparison, the extract of the regenerated plant showed maximum effect against *E.coli*(15.03±0.15), fairly high against *P.vulgaris*(13.9±0.15) *S. marcescens*(13.0±0.10) and *S.aureus* (12.0±0.15) and least against *K. pneumoniae*(8.06±0.05) as shown in table 1 and Figure 1.

The superior antibacterial activity of the regenerated plants over those of normal plants could be because of the greater quantities of the primary and secondary metabolites in the regenerated plants than in the normal plants<sup>44</sup>.

The results reveal that *Majorana hortensis* exhibits different degrees of antibacterial activity against the tested microorganisms, similar to the observations made by Ben et al.,<sup>40</sup>(2004) and Farooqi and Sreeramu<sup>36</sup>.

#### Conclusion:

The results of the present study showed that the aromatic plant *Majorana hortensis* exhibits antibacterial activities and the regenerates have better microbicidal properties than

the normal plants. The interest in microbicidal plants is ever increasing today since herbal pesticides and fungicides are harmless<sup>45</sup> and easily biodegradable<sup>46</sup>. The presence of antibacterial substances in higher plants is now well established<sup>47</sup> and they may prove to be more effective herbal protectants than the synthetic and commercial microbicides against a wide spectrum of pathogenic bacteria and fungi as these

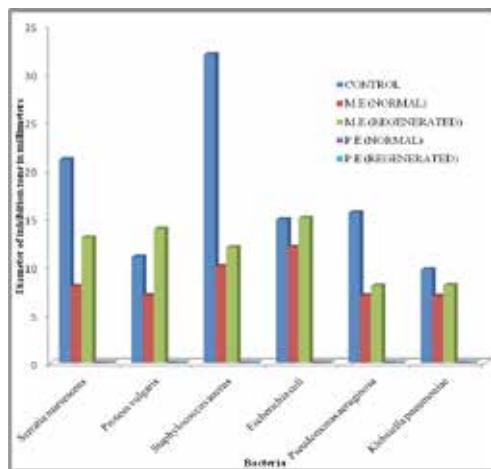
are eco-friendly and non-toxic.

Due to their antimicrobial activities the Phytomedicines can thus be safely used for the treatment of diseases in the indigenous systems of medicine or it can be the base for the development of a medicine, a natural blue print for the development of a drug<sup>48</sup>.

**Table-1. Antibacterial activity of methanolic and petroleum ether extracts of normal and regenerated plants of Majorana hortensis**

Bacteria	Diameter of the Inhibition zone(Mean±SD in mm.)				
	Methanol Extract		Petroleum Ether Extract		Control
	Normal	Regenerated	Normal	Regenerated	
Serratia marcescens	7.96±0.15	13.0±0.10	0	0	21.1±0.10
Proteus vulgaris	7.0±0.10	13.9±0.15	0	0	11.0±0.10
Staphylococcus aureus	10.0±0.15	12.0±0.10	0	0	32.0±0.10
Escherichia coli	12.0±0.15	15.03±0.15	0	0	14.9±0.05
Pseudomonas aeruginosa	7.0±0.10	8.0±0.10	0	0	15.6±0.05
Klebsiella pneumoniae	6.93±0.05	8.06±0.05	0	0	9.76±0.05

**Fig.1: Antibacterial activity of methanolic and petroleum ether extracts of normal and regenerated plants of Majorana hortensis**



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