

**DEVELOPMENT AND EVALUATION OF CHEMOTHERAPEUTIC POLY HERBAL  
COMBINATION FROM ERITREAN MEDICINAL PLANTS**

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**Summary**

To evaluate the antimicrobial activity of leaf and root extracts of *Ricinus communis*, *Vernonia amygdalina* and *Jasminium floribundum*, various pathogenic cultures viz *staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans* were used. Inhibition of organism's growth was investigated using disk diffusion methods. The chloroform crude extracts of *Vernonia amygdalina* and *Jasminium floribundum* showed significant antibacterial activity. However, none of the combined extracts showed higher activity than the individual extracts. From this it may be concluded that combination of these extracts less effective than using the individual extract against antimicrobial treatment. On the basis of the results there may be an antagonizing effect in between the extracts.

**Key words:** *Ricinus communis*, *Vernonia amygdalina* and *Jasminium floribundum*, Poly Herbal

**Introduction**

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants [1]. Therefore, such plants should be investigated to better understand their properties, safety and efficacy. The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments. Herbal medicine has been widely used and formed an integral part of primary health care in Eritrea. Traditional medical practitioners in Eritrea use a variety of herbal preparations to treat different kinds of microbial diseases. Also over the years, the World Health Organization advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and nonmicrobial origins [2].

In view of this, the present study has been designed to investigate the antimicrobial potential of three medicinal plants commonly found in Eritrea. The plants selected for the present investigation as the following:

*Ricinus communis* is a species of flowering plant in the spurge family, *Euphorbiaceae*. It belongs to a monotypic genus, *Ricinus*, and *sub tribe, Riciniinae*. The seeds of the plant contain between 40% and 60% oil that is rich in triglycerides, mainly ricinolein [3]. *Vernonia amygdalina*, a member of the *Asteraceae* family, is a small shrub that grows in the tropical Africa. It is commonly called bitter leaf. The leaves may be consumed either as a vegetable (macerated leaves in soups) or aqueous extracts as tonics for the treatment of various illnesses. [4, 5] *Jasminum floribundum* of the natural order oleaceae, which contains about 150 species, mostly natives of the warmer regions of the Old World. [6]

## Materials and Methods

### **Collection of Plant material**

The leaves of *Ricinus communis* and *Vernonia amygdalina* was collected from Asmara and nearby villages, while the roots of *Jasminium floribundum* was collected from Mai Nefhi, Eritrea.

### **Preparation of different extractives**

The 25g of each plant sample were immersed in chloroform, ethanol and water separately and were kept at 150 RPM over 24 hours on electric shaker. Samples were filtered using a vacuum pump device and the residues were re-immersed separately and shaken at 150 RPM for further 24 hours. After finishing the extraction process, the solvents were removed under reduced pressure using a rotary vapor, the yield obtained are given in Table 1. The extracts were stored in a clean dry glass bottle for 1 week at 5°C for further use for antimicrobial activity.

### **Antimicrobial activity**

#### **Microbial culture**

The bacterial and fungal cultures used were *staphylococcus aureus* ATCC 6538, and *Pseudomonas aeruginosa* ATCC 16145 and *Bacillus subtilis* and *Candida albicans* (clinical sample) was also used. These culture were collected from Medicinal plants drug discovery research centre (MPDDRC) Asmara, Eritrea and were maintained in a nutrient slant test tube at 4 °C.

#### **Culture media and antibiotics**

Müller-Hinton Agar (Merck) was used as a culture media. Gentamycin (Himedia) 10µg/disc and Co-trimoxazole (Himedia) 25µg/disc were used as a standard.

#### **Disk diffusion method**

The disc diffusion method was used to determine *in vitro* antimicrobial activity of the extracts and different combinations [6]. The cultures were subculture in agar medium and incubated at 37°C for overnight and from this, the spore suspension was prepared and standardized using 0.5 Mac Farland turbidity standards and 100µl of suspension was plated on agar medium using sterile swab, the organisms were uniformly inoculated into quality controlled Muller Hinton agar. Sterile empty discs (Hi-media) were allowed to soak and absorb the test samples for 24 hours before draining off the excess and drying in the oven at 60 °C [7, 8]. These discs were placed on the agar plates against

the control (solvents) and standard. Plates were incubated at 37<sup>0</sup>C for overnight and observed for the zone of inhibition. The tests were conducted in triplicate and average value was considered.

### **Results and discussion**

With the current spread of antibiotic resistance and challenges confronted with by medical practitioners in the treatment of infectious diseases, proper attention should be given to such plants to gather the potential antimicrobial benefits inherent in them. Among all the extract, chloroform and ethanol extracts of *Vernonia amygdalina* showed maximum activity. Water extract did not show any activity except *Ricinus communis* extract. *Bacillus* was the most inhibited microorganisms; it was inhibited by the ethanol and chloroform extract of all plants. *Pseudomonas aeruginosa* was the least inhibited bacteria, and showed resistance to crude extract of all plants. Root extract of *Jasminium floribundum* was found moderately active. Results are dose dependent, with increasing concentration of each extracts there was a general increase in the antimicrobial activity. With ethanol and chloroform extracts of *Jasminium floribundum*, very low activity was shown at lower concentration but significant increase at double concentration; and with similar extracts of *Vernonia amygdalina* no activity was seen at lower concentration but showed an astonishing high activity at doubled concentration (Fig 1). In the bi-mixture combination, none of the combined extracts showed higher activity than the individual extracts (Fig 2). This may conclude that combination of these extracts is less effective than using the extract in isolation against antibacterial treatment.

### **Conclusion**

Our results allow us to conclude that the chloroform crude extracts of *Vernonia amygdalina* and *Jasminium floribundum* shows significant anti bacterial activity. In addition, *Vernonia amygdalina* extracts are very active and hence this supports other studies and evidences in its surprising use in various infectious diseases like wound infection. In the bi-mixture combination, none of the combined extracts showed higher activity than the individual extracts (Fig 2). This may conclude that combination of these extracts is less effective than using the extract in isolation against antibacterial treatment. This might explain that there is an antagonizing effect in among the extracts. However, negative results do not indicate the absence of bioactive constituents, nor that the plant is inactive. Active compound(s) may be present in insufficient quantities in the crude extracts, hence to reveal its activity higher dose may be required. Lack of activity may thus be proven by using large doses. Alternatively, if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects on the positive bioactive agents. In addition, with no antibacterial activity, extracts may be active against other bacterial species which were not tested. So, although many actual antimicrobial ingredients are extracted and identified, still further researches and tests is required, in particular to assure their *in-vivo* effectiveness. Besides, any toxic effects on human and animal tissues should be investigated accordingly.

**Table 1: Yield percentage of different plant extractives**

Plants	Ethanol extract in % w/w	Chloroform extract % w/w	Water extract % w/w
<i>Ricinus communis</i>	3.04	3.84	3.40
<i>Vernonia amygdalina</i>	4.11	3.66	6.72
<i>Jasminium floribundum</i>	3.92	1.93	1.35

**Figure 1: Antimicrobial activity of different plant extracts**

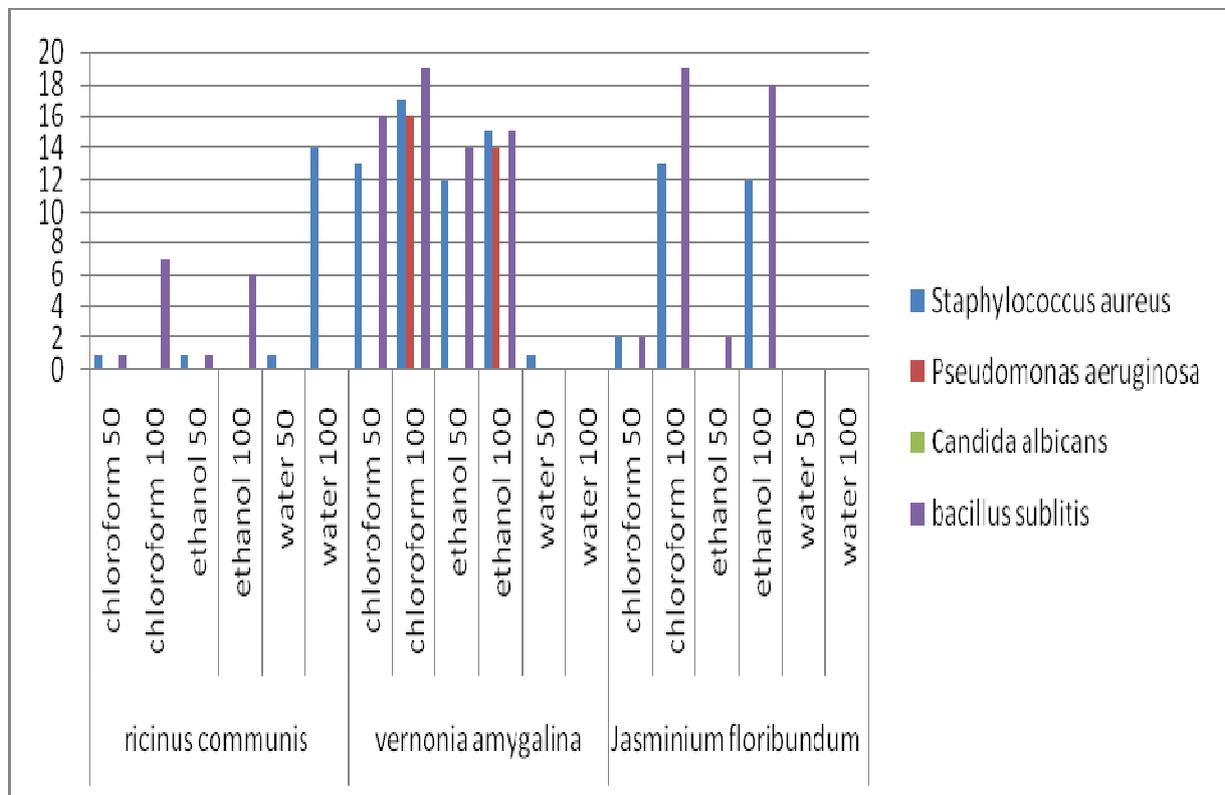


Figure 2: Antimicrobial activity of various combinations from active extracts

