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Comparative Evaluation of Poly Herbal Formulation from Traditionally Used Eritrean Medicinal Plants against Pathogenic Microorganisms

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JJK and NM designed the study. Authors JJK and AK wrote the protocol and wrote the first draft of the manuscript. Authors MK, MS, MT and SM managed the experimental work and screening. Author DM managed the literature searches and supervision of extraction process. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The present study was taken to investigate the *in vitro* anti-microbial activity of Poly herbal formulation (PHF) of *Schinus molle, Rhamnus prinoides*, *Grewia ferruginea, Achyranthes aspera* against different human pathogenic strains.
Methods: Leaves of selected plants were extracted by using Ethanol-Aqueous solution (70:30). The antimicrobial activities of different plants singly as well as various combined formulations were screened using agar well diffusion assay.
Results and Discussion: All Poly herbal formulation showed zone of inhibition ranged from 23-40 mm against *S. aureus* as well as it was found to be susceptible to all the selected plant extracts when tested as single plant extract. In antifungal screening, the combined extract of *Schinus molle*

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and *Grewia ferruginea* showed significant zone of inhibition against tested fungi when these two extracts were combined in the ratio of 1:1. *C. albicans* also showed susceptibility when all the four plant extracts were combined in equal ratio. While *E. coli* was found resistant to all the single and combined forms of the selected plant extracts.

Conclusion: Based on comparison aspect of antimicrobial activity of the plant extracts it can be concluded that combined plant extracts overcome the problem of resistance towards antimicrobial effect of a single plant extract or purified drug. Results also conclude that the diseases or infections caused by *S. aureus* and *C. albicans* can be treated by preparing drugs from these combinations of plants.

Keywords: Schinus molle; Rhamnus prinoides; Grewia ferruginea; Achyranthes aspera; antimicrobial; poly herbal combination.

1. INTRODUCTION

An old proverb in the Geez language– *e'tsyqietle'tsy ehieyw* ('a herb kills, a herb cures') personifies the rich, age-long knowledge of the Eritrean society on the curative powers of plants. Eritrean medicinal plants have been investigated on various aspects of disease curing drugs. Side effects and expenses associated with allopathic drugs have provoked the need for research into drugs which are without the side effects, especially those belonging to the traditional systems of medicine [1]. Research emphasis has been directed towards herbal drugs either in single or in combination having specific diagnostic and therapeutic principles. Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals [2]. It is estimated that 80% of the global population rely on plant derived medicines to address their health care needs. Medicinal plants are economically important major source for drug production [3].

The occurrence of resistant pathogenic strains is one of the major problems in the field of medicine and due to this reason the production of new drugs is needed to deal with the emergence of these strains. In order to achieve this, new drugs or new active compounds that can enhance the activity of existing drugs are needed [4].

In view of above information the present study has been undertaken to assess the antimicrobial activity of Poly herbal formulation containing bioactive compounds from different plants viz. *Schinus molle* L. varareira (L.) DC; *Rhamnus prinoides* L'Her; *Grewia ferruginea* Hochst. ex. & *Achyranthes aspera* var. indica L.

Schinus molle L. varareira (L.) DC *(Local name- Berbere tselim* Family-*Anacardiaceae)* is an ever- green tree 3-15 m in height. The tree's pendulant, informal branches and clusters of red-rose fruits add to its value as an ornamental. When fermented, the fruits make refreshing alcoholic drink known as chicha de molle. The dried fruits are boiled to produce honey de molle which, when fermented, yields a vinegar-like substance. The fresh leaves, bark, and roots of the tree are used to alleviate or cure rheumatism, bronchial infections, high blood pressure, ulcers, tumors, anxiety, and inflammations of the skin [5]. Phytochemically, the fruits and leaves part have flavonoids, quercetin and musizin. *Schinus molle* has antimicrobial activity against *S. aureus* and *C. albicans* [6].

Rhamnus prinoides (Local name- Giesso, Family-Rhamnaceae, Plants are scrambling up to 9 m in height usually spineless, with no buttresses. Leaves are alternate, simple, and not deciduous. Phytochemically, the leaves contain flavonoids, quercetin, musizin, rhamnazin, geshoidin and rhamnocitrin. Plant has antimicrobial activity against *E. coli* and *S. aureus*. *Rhamnus prinoides* has many traditional uses in different countries such as in Eritrea and Ethiopia for the traditional drink which is Swa or Tella [7].

Grewia ferruginea (Local name-Tsenqua, Family-*Tilliaceae) is* scrambling, up to 2 m tall; young branches densely ferruginous- hairy. Leaves are ovate to broadly elliptic. Phytochemical studies shows plant posses' tri-terpenoids, fatty components, flavonoids, steroids, saponins and tannins. The plant is believed to have anti-parasitic, anti-bacterial, as well as anti-fungal activity. The traditional healers believed that the leaves used for the evil spirit [8].

*Achyranthes aspera (Local name- Mechelo, Family- Amaranthaceae) is a*n erect herb, 0.3-1meter high with stiff branches terete or absolutely quadrangular, with few leaves. Phyto-chemically, the leaf extracts contain secondary metabolites such as flavonoid and glucoside [9].

The leaves extract have antimicrobial activity against *E. coli* and *S. aureus*. Traditionally, the plant is used in asthma, cough and for bites of poisonous snakes and reptiles. It is pungent, anti phlegmatic, anti periodic, diuretic, purgative and laxative, useful in oedema, dropsy and piles, boils and eruptions of skin etc. Crushed plant is boiled in water and is used in pneumonia. Infusion of the root is a mild astringent in bowel complaints. The plant is useful in liver complaints, rheumatism, scabies and other skin diseases. It also possesses tranquillizing properties [10].

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Four plants (*Achyranthes aspera, Rhamnus prinoides*, *Schinus molle* and *Grewia ferruginea)* were selected and collected from Asmara, Adiqe and Halhale cities of Eritrea, in February 2013.The leaves of the selected plants were authenticated by Dr. Ghebrehiwet, Botanist, Eritrean Institute Technology (EIT), Mai-Nefhi, Asmara, Eritrea. The voucher specimens (Aa-010, Rp-011, Sm- 012 and Gf-013 respectively) were deposited in the department of Clinical Laboratory Sciences, School of Allied Health Profession, ACHS, Asmara, Eritrea (Fig. 1).

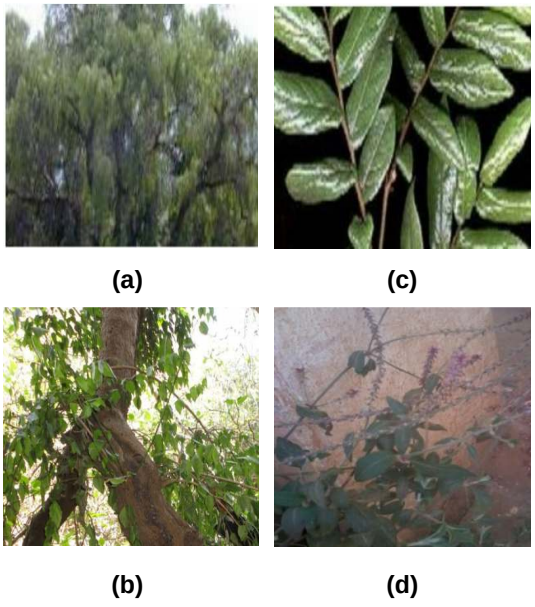


Fig. 1. a) *Schinus molle,* b) *Grewia ferruginea,* c) *Rhamnus prinoides*, d) *Achyranthes aspera*

2.2 Preparation of Plant Extract

Plants materials (leaves) were shade dried and coarsely powdered with the help of a grinder followed by sieving. Weighed material (48 g) was subjected to cold extraction method (maceration) by using Ethanol -Aqueous solution (70:30). The extracts were concentrated under reduced pressure at 40℃ which were stored in clean glass bottle at 4℃ for further antimicrobial screening. All the chemicals and reagents used for the experiments were of analytical grade.

2.3 Preparation of Various Poly Herbal Formulations

Seven Poly herbal formulations were prepared from the extracts of selected plants by taking equal amount of each extract (Table 1).

2.4 Preparation of Dilution of the Different Combinations

In all the poly herbal combinations, the solvents used for the dilution purpose were 70% ethanol and 30% distilled water. The following formula or equation has been developed to prepare the various combinations. Volume of the diluents was calculated using the following formula and tabulated in Table 2.

$$Y = X \div Z$$

Where:

X indicates weight of the combination (mg)
Y indicates volume of the diluents (ml)
Z indicates the dilution (mg/ml)

2.5 Antimicrobial Study

2.5.1 Pathogenic microbial strains

The bacterial and fungal strains used in the present study were *Escherichia coli* (ATCC 25922) *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 10231). All microbial strains were procured from National health Laboratory (NHL) Asmara, Eritrea. Muller Hinton Agar/ Broth and potato dextrose agar mediums were used to grow the bacterial and fungal strains respectively.

2.5.1.1 Agar well method

The antimicrobial screening was performed by using agar well method to determine the zone of inhibition [11]. Wells were made in seeded agar plate using a pre-sterilized cork borer (8 mm diameter) at equidistance and the test sample 20 micro litre was then introduced directly into these walls.

Table 1. Various combinations from different plants

No. of combination	Combination of plant extracts	Amount combined
1	*Schinus molle & Rhamnus prinoides*	50% each
2	*Schinus molle & Grewia ferruginea*	50% each
3	*Schinus molle & Achyranthes aspera*	50% each
4	*Rhamnus prinoides & Grewia ferruginea*	50% each
5	*Rhamnus prinoides & Achyranthes aspera*	50% each
6	*Grewia ferruginea & Achyranthes aspera*	50% each
7	*Schinus molle, Rhamnus prinoides, Grewia ferruginea & Achyranthes aspera*	25% each

Table 2. Preparation of combinations at different concentrations

	Combination	Concentration for antimicrobial study (mg/ml)		
		Amount mixed for 500 mg/ml	Amount mixed for 250 mg/ml	Amount mixed for 125 mg/ml
Plant extracts combinations	A+B	250 mg each	125 mg each	62.5 mg each
	A+C	250 mg each	125 mg each	62.5 mg each
	A+D	250 mg each	125 mg each	62.5 mg each
	B+C	250 mg each	125 mg each	62.5 mg each
	B+D	250 mg each	125 mg each	62.5 mg each
	C+D	250 mg each	125 mg each	62.5 mg each
	A+B+C+D	125 mg each	62.5 mg each	31.25 mg each
Volume of the diluents	Ethanol	0.7 ml	1.4 ml	2.8 ml
	Distilled water	0.3 ml	0.6 ml	1.2 ml

A=Schinus molle, B = Rhamnus prinoides, C= Grewia ferruginea, D= Achyranthes aspera

3. RESULTS AND DISCUSSION

3.1 Yield Percentage of Different Extractives

Yield percentage of different extracts were calculated and summarized in Table 3. Maximum yield was obtained from the leaves of *Rhamnus prinoides* while the least percentage was calculated from the *Grewia ferruginea* leaves.

3.2 Antimicrobial Study

3.2.1 Determination of zone of inhibition

The ethanol aqueous extract of each selected plant showed effective antimicrobial activity against *S. aureus* while *E. coli* was resistant to all the plant extracts. *C. albicans* was susceptible to the extracts of *Schinus molle* and *Grewia ferruginea* (Table 4).

Table 3. Yield percentage (w/w) of various plant extractives

Plant material	Solvent used	Weight of the material (g)	Weight of the extract (g)	Yield (w/w) %
Schinus molle (Berberetselim)	70% ethanol	48	12	25
Rhamnus prinoides (Gesso)	70% ethanol	48	14.68	30.6
Grewia ferruginea (Tsinquea)	70% ethanol	48	8.57	18
Achyranthes aspera (Machello)	70 % ethanol	48	10.05	21

Table 4. Antimicrobial activity of individual plant extracts

Plant extracts	Concentration (mg/ml)	Agar well-method (mm)		
		S. aureus	*E. coli*	*C. albicans*
A	500	16	No	12
	250	12	No	9
	125	9	No	7
B	500	16	No	No
	250	13	No	No
	125	11	No	No
C	500	13	No	11
	250	10	No	9
	125	7	No	6
D	500	17	No	No
	250	10	No	No
	125	8	No	No

A=Schinus molle, B =Rhamnus prinoides, C= Grewia ferruginea, D= Achyranthes aspera
Note: All the results were compared with standard drugs Amoxicillin and Fluconazole

Table 5. Antimicrobial activity of plant extracts in various combinations

PHC	Concentration (mg/ml)	Agar well method (mm)		
		S. aureus	*E. coli*	*C. albicans*
AB	500	39	No	No
	250	32	No	No
	125	29	No	No
AC	500	17	No	14
	250	16	No	11
	125	15	No	9
AD	500	36	No	No
	250	30	No	No
	125	29	No	No
BC	500	40	No	No
	250	34	No	No
	125	23	No	No
BD	500	30	No	No
	250	28	No	No
	125	27	No	No
CD	500	37	No	No
	250	36	No	No
	125	27	No	No
ABCD	500	32	No	17
	250	29	No	16
	125	26	No	15

PHC = Poly herbal combination, No= No zone recorded
A=Schinus molle, B=Rhamnus prinoides, C= Grewia ferruginea, D= Achyranthes aspera
Note: All the results were compared with standard drugs Amoxicillin and Fluconazole

Different poly herbal combinations of the selected plants were also made by mixing different plant extracts (Tables 1 and 2). All the extracts were combined in the equal ratio. These different combinations were tested for antimicrobial activity (Table 5).

Results showed that all the combinations when tested against *S. aureus* showed enhanced antimicrobial activity as compared to individual plant activity. As the zone of inhibition ranged from 23-40 mm in Poly herbal formulation as compare to the zone of inhibition of single active plant ranged from 8- 17 mm (Fig. 2).

C. albicans was susceptible to the combination of extracts of *Schinus molle* and *Grewia ferruginea* when combined in the ratio of 1:1. *C. albicans*

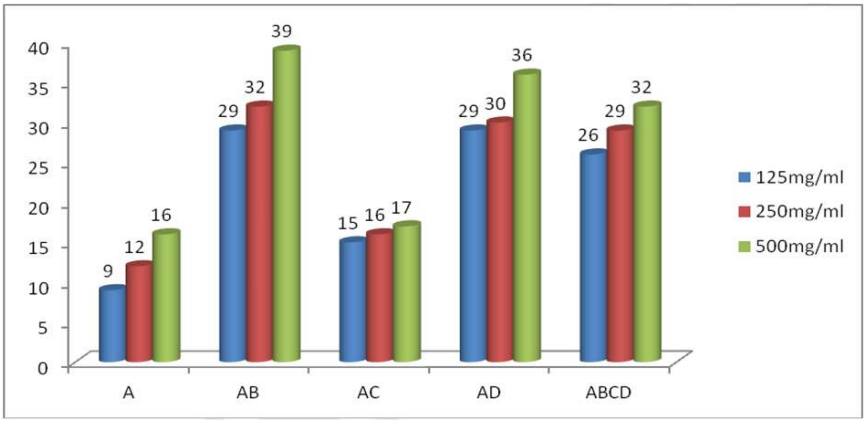
also showed susceptibility when all the four plant extracts were combined. While *E. coli* was resistant to all the combinations of plant extracts in all the concentration.

3.2.2 Comparative antimicrobial study of individual plant with various poly herbal combinations

When comparative study was done between individual and various poly herbal combinations, it was found that the antimicrobial activity of the selected plants increased significantly in various combinations compared to the active single plant (Graphs 1-6). All the results were compared with standard drug Amoxicillin.

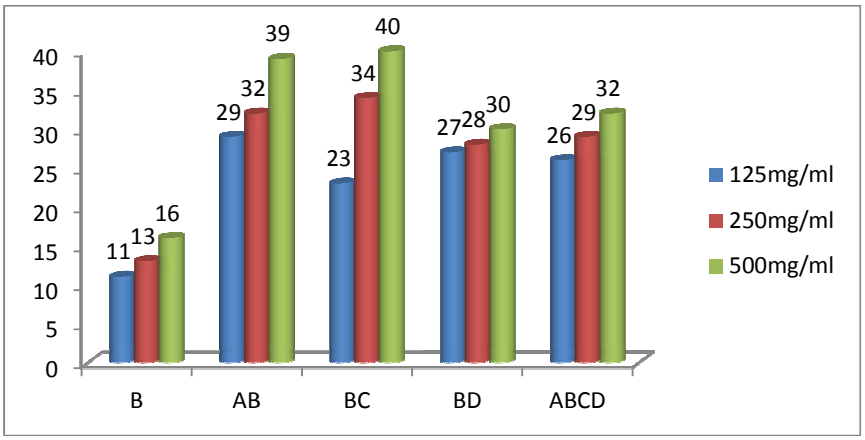
From Table 5 it can be observed that when various combinations of plant extracts were tested, the BC (*Rhamnus prinoides* combined with *Grewia ferruginea*) combination showed zone of inhibition of 40 mm, 34 mm and 23 mm respectively at 500 mg/ml, 250 mg/ml and 125 mg/ml concentrations against *S. aureus*. Similarly when *Rhamnus prinoides* (B) was combined with *Schinus molle* (A) extract in equal ratio and tested against *S. aureus* then the zone of inhibition was 39 mm, 32 mm and 29 mm respectively at 500 mg/ml, 250 mg/ml and 125 mg/ml concentration (Fig. 2).

Likewise when *Rhamnus prinoides*(B) was combined with *Achyranthes aspera* (D) extract in equal ratio and tested against *S. aureus* then the zone of inhibition was found to be 30 mm, 28 mm and 27 mm respectively at 500 mg/ml, 250 mg/ml and 125 mg/ml concentration (Fig. 2).



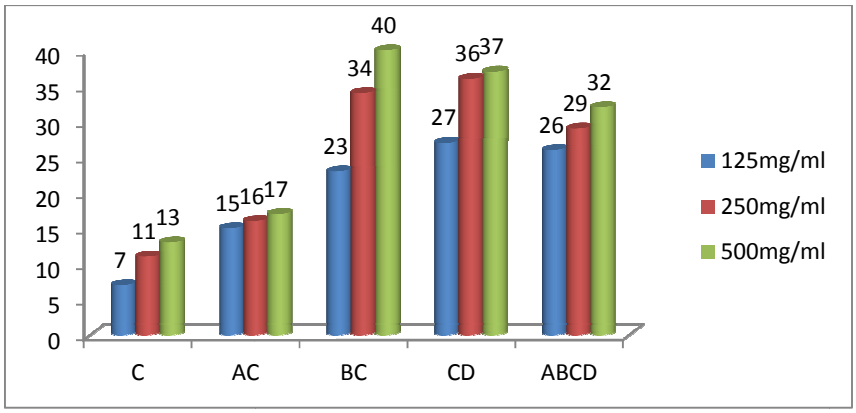
Graph 1. Graphical representation of antimicrobial activity of individual and combinations of *Schinus molle* at different concentrations against *S. aureus*

A=Schinus molle, B =Rhamnus prinoides, C= Grewia ferruginea, D= Achyranthes aspera



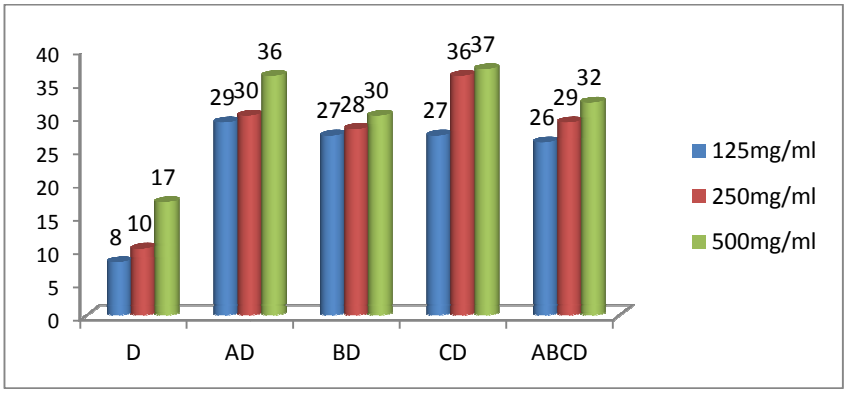
Graph 2. Graphical representation of Antimicrobial activity of individual and combinations of *Rhamnus prinoides* at different concentrations against *S. aureus*

A=Schinus molle, B =Rhamnus prinoides, C= Grewia ferruginea, D= Achyranthes aspera



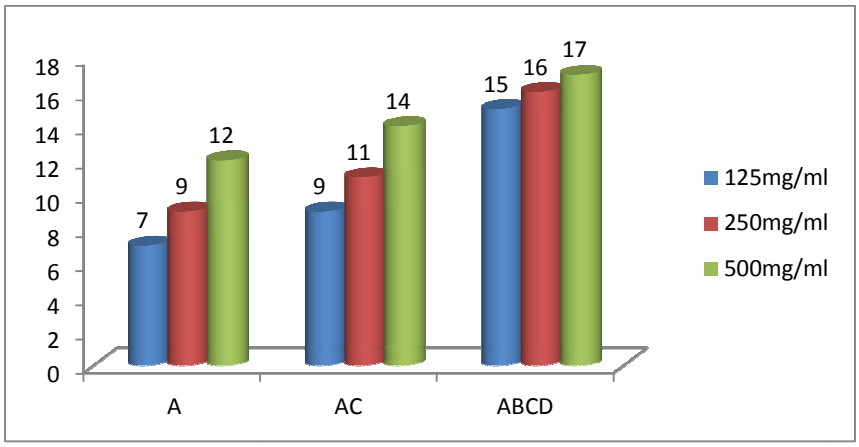
Graph 3. Graphical representation of Antimicrobial activity of individual and combinations of *Grewia ferruginea* at different concentrations against *S. aureus*

A=Schinus molle, B =Rhamnus prinoides, C= Grewia ferruginea, D= Achyranthes aspera
Note: All the results were compared with standard drug Amoxicillin



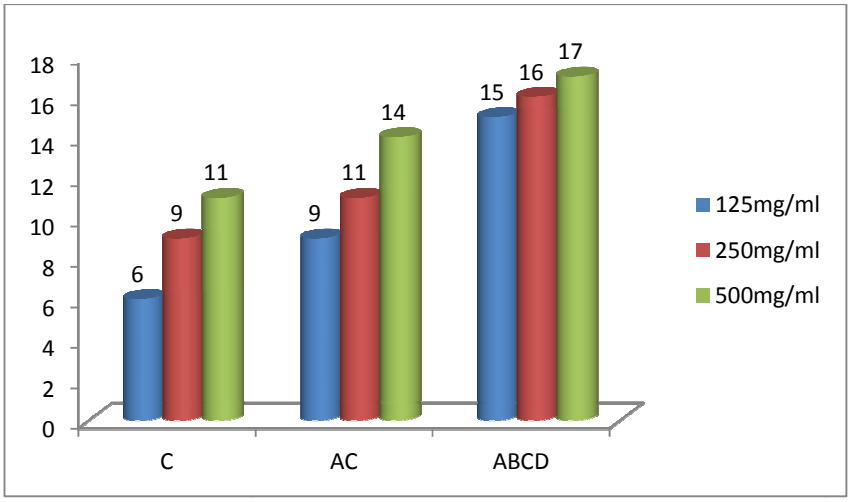
Graph 4. Graphical representation of antimicrobial activity of individual and combinations of *Achyranthes aspera* at different concentrations against *S. aureus*

A=Schinus molle, B =Rhamnus prinoides, C= Grewia ferruginea, D= Achyranthes aspera
Note: All the results were compared with standard drug Amoxicillin



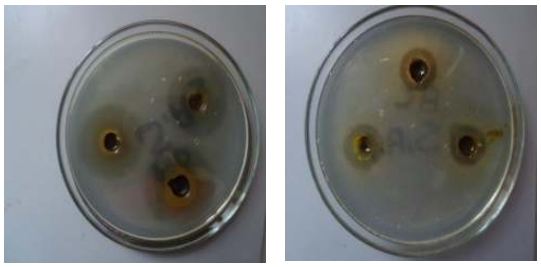
Graph 5. Graphical representation of antimicrobial activity of single and combinations of *Schinus molle* at different concentrations against *C. albicans*

A=Schinus molle, B =Rhamnus prinoides, C= Grewia ferruginea, D= Achyranthes aspera
Note: All the results were compared with standard drug Fluconazole



Graph 6. Graphical representation of Antimicrobial activity of single and combinations form of *Grewia ferruginea* at different concentrations against *C. albicans*

A=Schinus molle, B =Rhamnus prinoides, C= Grewia ferruginea, D= Achyranthes aspera
Note: All the results were compared with standard drugs Fluconazole



(a) **(b)**

Fig. 2. a) Zone of inhibition shown by combination of *Rhamnus prinoides* with *Grewia ferruginea* against *S. aureus*
b) Zone of inhibition shown by *Schinus molle* against *S. aureus*

From the data obtained from Table 5 it can be predicted that when *Rhamnus prinoides* (B) was combined with *Schinus molle* (A) or with *Grewia ferruginea* (C) it showed a more effective antimicrobial activity against *S. aureus*. The extract of *Grewia ferruginea* (C) when combined with *Achyranthes aspera* (D) and tested against *S. aureus* showed a zone of inhibition 37 mm (500 mg/ml concentration) but when *Grewia ferruginea* (C) combined with *Schinus molle* (A) a zone of inhibition of 17 mm was obtained (500 mg/ml concentration). This shows that *Grewia ferruginea* (C) extract antimicrobial efficacy was enhanced when combined with *Achyranthes aspera* (D) extract or *Rhamnus prinoides* (B) extract. The *C. albicans* was susceptible to the extracts of *Schinus molle* (A) and *Grewia ferruginea* (C) as well as when these two extracts were combined (AC) in the equal ratio, the antimicrobial activity was increased as a zone of inhibition of 14 mm was obtained. *C. albicans* also showed susceptibility when all the four plant extracts were combined (ABCD) with an inhibition zone of 17 mm.

From the data presented in graphs 1-6 it can be assumed that all plants showed different compatibility when mixed to form different Poly herbal formulations.

In contrast to the studies conducted by A. A. Azmi et al. [12] in 2010 and S. A. Hussain et al. [13] in 2011 on different poly herbal formulations, the results from both the studies indicates significant increase in antimicrobial activity against test microorganisms. Likewise the present study also showed increased *in-vitro* antimicrobial activity in various poly herbal combinations.

4. CONCLUSIONS

Based on comparison aspect of antimicrobial activity of the plant extracts it can be concluded that combined plant extracts overcome the problem of resistance towards antimicrobial effect of a single plant extract or purified drug. Results also conclude that the diseases or infections caused by *S. aureus* and *C. albicans* can be treated by preparing drugs from these combinations of plants. This study will

furthermore promote the discovery of new alternative drugs to currently being used. It will also serve to broaden the studies that have so far been conducted in Eritrea, especially on those regionally endemic plants with antimicrobial activities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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