

The Combined Effect of Two Aqueous Extracts on the Growth of *Trichomonas vaginalis*, *in vitro*

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SUMMARY: In this study a trial was performed to determine the effect of a combination of two aqueous extracts of two medicinal plants namely the violet, *Viola odorata*, and the rue, *Ruta graveolens*, with concentrations of 0.15625, 0.3125, 10-20 mg/cm³ on the growth of *Trichomonas vaginalis* cultured in (CM161) medium during periods of 24, 48, 72, and 96 hrs. The results showed that there is a variation of inhibition. Complete inhibition was seen with a concentration 10 mg/cm³ for 48 hrs. During the 96 hr. Period, 81% inhibition was achieved at a concentration of 0.3125mg /cm³, and 75% at a concentration of 0.15625mg/cm³. A variety of interaction was observed in combination of violet and rue. Significant synergism was achieved at a concentration of 20 mg/cm³, and a suggestive synergism was achieved with 10 mg /cm³ In addition, partial antagonism occurred at a concentration of 0.15625mg /cm³ and an antagonism at 0.3125mg /cm³ was achieved.

Key Words: *Trichomonas vaginalis*, *in vitro*

Trichomonas vaginalis'in Gelişimine İki Sulu Özü Birlikte Olan Etkisi, *in vitro* Çalışma

ÖZET: Bu çalışmada menekşe, *Viola odorata*, ve sedefotu, *Ruta graveolens*, adlı iki bitkinin sulu özünün birlikte (0.15625, 0.3125, 10-20 mg/cm³ lük yoğunluklarda) CM161 besiyerinde *Trichomonas vaginalis* 'in gelişmesi üzerine yaptığı etki (24, 48, 72, ve 96 saatlik dönemlerde) incelendi. Alınan sonuçlar inhibisyonda farklılıklar olduğunu gösterdi. 48 saatte 10 mg/cm³ lük yoğunluk ile tam bir inhibisyon görüldü. 96 saatte, 0.3125 mg/cm³ lük yoğunluk ile %81 ve 0.15625 mg/cm³ lük yoğunluk ile %75 oranında inhibisyonlar elde edildi. Menekşe ve sedefotu birleşiminde çeşitli etkileşimler gözlemlendi. Anlamlı bir sinerji 20 mg/cm³ lük yoğunlukta ve olası bir sinerji 10 mg/cm³ lük yoğunlukta elde edildi. Ayrıca, kısmi bir antagonizm 0.15625 mg /cm³ lük yoğunlukta ve tam bir antagonizm 0.3125mg /cm³ lük yoğunlukta oluştu.

Anahtar Sözcükler. *Trichomonas vaginalis*, *in vitro*

INTRODUCTION

Trichomonas vaginalis is a cosmopolitan species, found in reproductive tract of both men and women. It lives in the vagina and urethra of women and in the men prostate, seminal vesicles and transmitted primarily by sexual intercourse (11). Cultivation is useful process in showing growth phases and a route for confirming diagnosis (4, 7).

Oral drugs such as metronidazole, usually cure infection in about five days (6, 12). Suppositories and douches are useful in promoting an acid pH of the vagina (13). Furazolidone was the best alternative for strain resistant to flagyl (9). Several trials were designed to select some medicinal plants to screen

their effect as growth inhibitors to the parasitic protozoa (3).

In Iraq, the extract of *Myrtus commanis* was used and found that caused inhibition to *T. vaginalis* at pH=4.65 while at pH=6.0 failed to do so, while *Eucalyptus* extract at pH=5.35 showed a good therapeutic effect after 24 hrs, at a concentration of 0.05 gm/0.1 ml medium (5).

Recently, a trial was made to use extracts of violet and rue solely, and a significant inhibitory effect was noticed (1). In the present project, a combination effect of both these plants was tested to screen their effect whether synergetic or antagonistic.

MATERIALS AND METHODS

Specimens of *T.vaginalis* were collected from Hospitals by using swabs, cultured in Oxoid *Trihomonas* medium (CM161). The leaves of two plants violet, *Viola odorata* and

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rue, *Ruta graveolens*, collected, cleaned then dried without direct sunlight for 3-4 days (12). Aqueous extracts were obtained by mixing 40 gm of each plant powder (violet&rue) (10), with 160 cm³ of distilled water in the ratio of 1:4, crushed in porcelain mortar, the mixture was left for 24 hrs, at 4 °C, then filtered and centrifuged. The extract was lyophilized and then preserved in the deepfreezer (-20 °C) ready to be used. The interaction for the mixture of the two extracts was calculated (11).

RESULTS AND DISCUSSION

As shown in Table (1) the effect of combination of the two aqueous extracts of violet and rue, on the growth of *T. vaginalis* cultivated on medium CM161 for different periods.

It is proposed recently that there are different ways in which the molecules can pass through the plasma membrane of the animal cell (8), either facilitated transport such as the glucose and amino acids is facilitated by their reversible combination with carrier proteins which in some manner transport them through the plasma membrane. Or as in nerve or muscle cells and via sodium-potassium pump i.e. the active parts of these two plants combine alternatively with sodium ions and potassium ions. Or by the well known manner, phagocytosis or pinocytosis or receptor-mediated endocytosis, which more likely to happen in our experiments as solution was used. Use a receptor protein shaped in such a way that specific molecule can bind to it. A macromolecule that binds to a plasma membrane receptor is called a ligand.

Table 1. The effect of combination of the two aqueous extracts of the violet and rue, on the growth of *Trichomonas vaginalis* cultivated on medium (CM161) for different periods (parasite number 5×10^5)

| Dosage of treatment mg/cm ³ | Duration of treatment hrs. | | | | | | | |
|--|----------------------------|--------------|------|--------------|------|--------------|------|--------------|
| | 24 | | 48 | | 72 | | 96 | |
| | Mean | % inhibition | Mean | % inhibition | Mean | % inhibition | Mean | % inhibition |
| Control | 15.00 | 0 | 17.5 | 0 | 6.50 | 0 | 1.00 | 0 |
| 0.15625 | 4.58 | 69 | 7.00 | 60 | 3.33 | 49 | 0.25 | 75 |
| 0.3125 | 3.25 | 78 | 5.00 | 71 | 2.58 | 60 | 0.13 | 87 |
| 10 | 0.75 | 95 | 0.00 | 100 | 0.00 | 100 | 0.00 | 100 |
| 20 | 0.00 | 100 | 0.00 | 100 | 0.00 | 100 | 0.00 | 100 |

* Each value represent the mean for three replicates .

Complete inhibitory effect after combination was noticed at concentration of 20 mg/cm³ within 24hrs and at concentration of 10 mg/cm³ during 48hrs. This proved that had different degrees of inhibition of parasite's growth (2). As shown in Table (2) a variety degrees of interaction between the extract of violet and rue was recorded, at concentration of 20mg/cm³ significant synergism was achieved because the regression coefficient (r.c.) was less than 0.05 (10) and suggestive synergism was achieved at concentration of 10mg/cm³ because the r.c. was 0.38. Furthermore the partial antagonism was recorded at concentration of 0.15625 mg/cm³ because the r.c. was 1.9 and antagonism happened at concentration of 0.3125 mg/cm³ because the r.c. was more than 2 (10).

The binding of ligands to their receptors causes the receptors together at one location. This location is called a coated pit because there is a layer of fibrous protein, called clathrin, on the cytoplasmic side. Clathrin seems to somehow facilitate the formation of a vesicle. Once the vesicle is formed, the clathrin coat is released and the vesicle appears uncoated. The fate of the vesicle and its contents depends on the kind of ligand it contains. Obviously such ways of plants extracts taken to enter the parasite were not studied at such molecular level, therefore it is suggested to be done here in order to understand synergism found in the present research.

Table 2. The effect of combination of two extracts of both violet and rue on the regression coefficient number of *T. vaginalis* for the different periods

| Regression coefficient | Time | | | |
|--------------------------------------|------|-----|------|-----|
| | 24 | 48 | 72 | 96 |
| Concentration hrs. mg/c ³ | | | | |
| 0.15625 | 1.9 | 3.8 | 4.9 | 1.4 |
| 0.3125 | 2.6 | 8.5 | 11.0 | 0.0 |
| 10 | 0.38 | 0.0 | 0.0 | 0.0 |
| 20 | 0.0 | 0.0 | 0.0 | 0.0 |

Less than 0.05 =significant synergism, 0.05 –0.9 = suggestive synergism, 1 = additive, 1.1 –1.9 = partial antagonism, * More than 2 = antagonism

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