# Antimicrobial Study of Some Chemical Preparations for Vaginal Isolates

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## Abstract

**The aim of this study was identification of most common microorganisms of vaginitis with effect of Alum & Gentian violet on them.**

**This study was performed between Feb. 2013 and Juan. 2014, involving 215 married women at 15-49 years who attending TTH and private clinic in Tikrit city, they were examined and taking high vaginal swab. For diagnosis, used Amsel's criteria, Gram staining of vaginal discharge, culture, biochemical tests and API system.**

**Vulvovaginal candidiasis is most common 70(32.6%) than BV associated Gardnerella vaginalis 18(8.4%), in our distinct. Candida albicans represent 32.6%, while E. coli and S. aureus were 11.6% and 7.4% respectively. The history of abortion and PTL in those patients was at 1st and 3rd trimester of gestational age. By disc diffusion method showed resistance 100% to metronidazole. There was a significant strong correlation between Alum and Gentian violet at concentrations (1% to 4%) and the inhibition growth zone of G. vaginalis and C. albicans with statistically significant result at p-value <0.001.**

**Key words:** vaginitis, BV, *G. vaginalis, C. albicans*, alum, gentian violet.

## Introduction

Vaginitis is an inflammatory of the vaginal mucosa or infection of the vagina, is the most common gynecological condition encountered by physicians in the office, whose incidence appears to be increasing, as estimated that 75% of women will at least one episode of vaginitis (1). The two most common causes of infectious vaginitis are BV and yeast vaginitis. BV is accounting to 40-50% of cases (2). Candidiasis is well recognized type of vaginitis represented 75% of all women will once in their lifetime, Candida albicans represented 80% (3). Epidemiological studies revealed BV is not to be an exclusive STD but somehow caused by sex by increase the vaginal pH since semen is alkaline pH (4). Multiple sexual partners, having new sex partner and women who have sex with women, in who Candida vaginitis is most common seen. Increasing parity and frequency of having sex, also hormonal changes (Menes, pregnancy) and oral contraceptive use, antibiotic therapy, and foreign bodies in the vagina (tampons, IUDs) (5).

Although vaginitis is not serious condition but its potential squeal during reproductive age group in pregnant and non-pregnant women even in both developed and developing countries and resulted in improved diagnostic methods and treatment (6).

The worldwide increase in multidrug resistance of pathogenic bacteria has led to an urgent need for identifying an alternative strategies to counter bacterial infection. The latest research have been focused on identifying the potential antimicrobial agent from the natural resources. Natural products have been used for centuries in treating human diseases and they contain components of therapeutic value which are

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environmentally safer, easily available, alum and gentian violet have become a favorite antimicrobial and can therefore be used as a natural inhibiting the growth of the bacteria and fungus (7).

## Materials and Methods

**Patients:** The study population was 215 married women, at aged between 15-49 yr. old, who attending the outpatient of gynecological and obstetric clinic in Tikrit Teaching Hospital and private clinics in Tikrit province in the period from February 2013 to January 2014. Most of the patients were suffering from vaginal discharge, itching, bad odor, dysuria and dyspareunia. The patients with diabetes mellitus, received corticosteroids drug, malignancy, menstrual bleeding and received antimicrobial drugs less than one week, will be excluded. Control were 65.

Sample collection and treatment: By using unlubricated sterile Cusco's speculum was inserted into vaginal women and the lateral and posterior vaginal fornix were swabbed with two sterile cotton tipped applicators. One of the swab was for put in tube with sterile Amies transport media and transported to microbiological laboratory where cultured, and other swab be was for microscopic examination (wet mount and Gram stain). A pH strip placed in contact with the secretions on the speculum after it had been withdrawn measured vaginal pH. BV and AV was clinically diagnosed when presence homogeneous vaginal discharge and pH > 4.5. Amine test was performed by adding a drop of 10% KOH to the discharge on the used speculum (whiff test) (8).

**Morphological characteristics:**

**a. Gram's Stain:** Clue cells were diagnosed by Gram staining in which vaginal epithelial cell covered with gram variable short bacilli suggested BV [9, 10]. Microscopic exam of yeast as Gram positive large oval or budding yeast cells and the presence of no hyphae, which indicate *Candida glabrata*, not *Candida albicans*. *Candida glabrata* does not produce hyphae, only spores, which it is very resistant because of the biofilm it creates to protect itself.

**b. Cultural characteristics:** For culture, used selective media for *G.vaginalis* is Columbia blood agar base, with FD056, incubated anaerobically at 37 $^{O}$C/48 hour in candle jar to provide 5-10% $CO_2$ , which showed small pin-point colonies, translucent, β-hemolytic if human blood used, also both oxidase and catalase tests are negative [11]. *G.vaginalis* ferments carbohydrates such as dextrin, maltose, glucose, fructose, sucrose, ribose and starch. Also, streaked the Blood agar, Chocolate agar which were incubated in microaerophilic atmosphere while MacConkey's agar, Eosin methylene blue agar, Mannitol salt agar, Pseudomonas agar and Sabouraud's dextrose agar-Chloramphenicol incubated aerobically at 37 $^{O}$C/24 h., but fungi incubation may reach 72h.

**c. Biochemical tests:** Including catalase test, coagulase test (slide method), oxidase test, mannitol fermentation test, indole test, carbohydrates fermentation and gas production, citrate utilization test, urease production test, lipase test, phospholipase test, β– galactosidase test, protease test, MR/VP test, bacitracin differentiation test according to references [10, 12].

**d. API system:** **Api* Staph System was used for diagnosis of *Staphylococcus* and to differentiate between *S. aureus* and other coagulase negative *Staphylococci* and *Micrococcus spp.*

**Api* 20 Strep was used for diagnosis of *Streptococcus* and *Enterococcus spp.*, and other types of *Sterptococcus spp.*

**Api* Candida was used for diagnosis of *Candida spp.*

**e. RapID ™ NH ONE System:**

RapID™NH ONE System (remel) was used for diagnosis and identification of *Gardnerella vaginalis*.

**f. Germ tube test:** Small inoculum of yeast cells, which was obtained from an isolated colony, suspended in 0.5 ml of serum. The test tube was incubated at 37 $^{O}$C for no longer than three hrs. Then the drop of the suspension was placed on clean dry slide and examined under low power of light microscope for presence of germ tube, which was defined as appendage, which was half the width, and 3-4 times of the length of the yeast cell. The appearance of germ tube was indicated for *C. albicans* [13].

**Disk diffusion method (Kirby Bauer test):**

The susceptibility of *G. vaginalis* against various antibiotics were studied, using Kirby-Bauer method. The disk used 5 mm in disk diffusion method. On using aseptic technique of disk diffusion method, the inhibition zone was measured in mm. A sterile swab placed on the broth culture of *G.vaginalis*. The inoculate was transferred to Muller-Hinton agar plate by streaking in three direction. The plate left 5 minutes, and antibiotic discs were dispensed onto the agar using flame-sterilized forceps. Plates should be incubated at 37 $^{O}$C for 24-48 hours. The antibiotics and antimicrobials were used as metronidazole, cefotaxime, doxycycline and Penicillin G [10, 13].

**Evaluate the antimicrobial effect of Alum and Gentian violet:**

We used two chemical compounds or preparation as antiseptic agents against vaginal candidiasis (*Candida albicanus*) and BV associated *G. vaginalis* including:

1. Alum: (Aluminum potassium sulfate) KAl (SO4)2.12H2O
2. Gentian violet: Tris (4-(dimethylamine) phenyl) methylium chloride = $C_25N3H3OCl$.

Agar well diffusion method was used to evaluate, in vitro, the antimicrobial effect of the Alum and Gentian violet against *G.vaginalis* and *C.albicans*, by means of agar-well diffusion assay. Mueller Hinton agar (45 °C) were poured into sterile petri dishes. Then taking 50 µl from 0.5 McFarland turbidity of subculture cell suspensions (24-48 hr., in BHI) by micropipette and spread onto the surface of the agar plates in three direction with swab and left 5 minutes. After that, making wells (6mm diameter, and 4mm height) were bored using a sterile cork borer. Different concentrations of the test solutions were placed into the wells and the plates were incubated anaerobically for *G. vaginalis* while, aerobically for *C. albicans* at 37°C for 24 hr. The plates were removed from the incubator and were examined for the inhibition zone around each well (if present), by using the ruler minimum calibration: 1mm [92, 93, 94].

**Alum and Gentian violet solutions preparation:**

A stock solution of Alum was prepared by mixing (0.05, 0.1, 0.5, 1, 2, 3, and 4)g of each Alum and Gentian violet with 100 ml of sterile distilled water which made (0.05%, 0.1%, 0.5%, 1%, 2%, 3% and 4%) respectively. The Alum stocks solutions were sterilized by filtration through a millipore filter, the millipore diameter was 0.22 mm [14, 15, 16].

**Statistical analysis and data management:** Chi ($\chi^2$) square test of association was used. One–way ANOVA was used to compare means of numerical variables, and Pie Bar, and Scatter graph used to represent the data. P value of $\leq 0.05$ is significant.

## Results

The present study was carried out on 215 women with signs and symptoms of vaginitis. Their age ranged between (15–49) years. There was fungal or yeast vaginitis

predominance (46.5%) among patients other than BV was (8.37%), while AV was (39.0%). This study also included (65) women apparently healthy individuals considered as a healthy control (table1).

The number of obstetrical problems in patients with BV and vaginitis were presented in (table2). Abortion, which occur within first trimester, was higher number (18.6%), while preterm labor in third trimester and abortion in second trimester were (8.4% and 6.5%) respectively.

Table (3) showed *Candida albicans* was the main pathogen which isolated from vaginal swab culture of patient with the vaginitis (32.6%), which was significant when the P value < 0.001, *Gardnerella vaginalis* and *Eschrichia coli* were (8.4%) and (11.6%) respectively.

The susceptibilities of *G. vaginalis* against various (4) antibiotics and antimicrobials were studied as in (table 4), which showed that (18) bacteria isolates were 18(100%) resistance to metronidazole, also 15(83.3%) and 14(77.8%) resistance to penicillin G and doxycycline respectively. However, there was 10 (55.6%) sensitive to doxycycline and 8 (44.4%) resistant.

Table (5) show that there is a significant strong correlation between Alum concentration and the Inhibition zone (mm) of *G. vaginalis* 0.964, and the Inhibition zone (mm) of *C. albicans* 0.821.

Table (6)show that there is a significant strong correlation between Gentian violet concentration and the Inhibition zone (mm) of *G. vaginalis* 0.909, and the Inhibition zone (mm) of *C. albicans* 0.898.

Table (7) showed the inhibition effect of (alum in 0.1, 0.5, 1, 2, 3 and 4) gm/100 ml sterilized by a Millipore filter compared with gentian violet in same concentration for both *G. vaginalis* and *C. albicans*. From the results alum was stock's concentration (0.1gm/100 ml) did not show any inhibition efficiency toward *G. vaginalis* and *C. albicans* isolates and (0.5gm/100ml) of alum did not have inhibition efficiency toward *C. albicans* only, but it had inhibition effect against *G. vaginalis*. (1, 2, 3 and 4 gm/100 ml) had high inhibition efficiency against *G. vaginalis* and *C. albicans,* compared with gentian violet (0.1 gm/100 ml) did not have inhibition effect against both *G. vaginalis* and *C. albicans*. The gentian violet in concentration (0.5, 1, 2, 3 and 4 gm/100 ml) had inhibition effect against both *G. vaginalis* and *C. albicans*.

## Discussion

Vaginitis is still a major cause of morbidity, and are not reportable diseases; therefore, accurate estimates of incidence are unavailable [17]. The diagnosis methods of BV and *Candida* vaginitis depended on the acuity of the clinician. The lack of standardized and diagnostic tools lead to misdiagnosis and consequently incorrect treatment [18]. The present study showed vaginitis, was caused by yeast infection, prominent 46.5% than BV associated *G. vaginalis* 8.3%, while AV was 39%. These results were significant, (Table 1). These agreed with [19] reported BV associated *G.vaginalis* incidence 5.2% in non- pregnant in Baghdad, also [20], mentioned the prevalence of BV was 7% and BV associated *G.vaginalis* was 7.7% in Basrah, while in AL-Diwaniya city [21], reported 28.6% women affected by BV and 93.7% of them with BV associated *G.vaginalis,* as well as reported 32.6% with other vaginitis like Candidiasis. In Babylon province [22] represent out of 105 vaginal sample 97 was infected with *Candida* vaginitis. Also, the present study accepted with [18], which showed 10-20% of women infected with BV and approximately 75% vaginal yeast infection. This difference in incidence of BV associated *G.vaginalis* may be attributed to the preservative relationships in the Iraqi society, in addition to that, the fact that most women in these studies were single and possibly had multiple sexual partners [23], at the same time *G. vaginalis* colonizes uncircumcised men more frequently [24]. The

present study showed women with vaginitis and BV had previous obstetrical problems such as abortion in the first trimester were 40(18.6%) case out of 215 patients, then in third trimester including PTL and still birth about 18(8.37%), but less in second trimester including 14(6.5%) case. The result accepted with [25, 26], who found 95% of premature rupture membrane and PTL has BV, and treatment of BV will reduce premature rupture membrane with PTL, (table 2).

The present study showed *Candida albicans* was most common microorganism 32.5% which causes *Candida* vaginitis, while BV associated *G.vaginalis* was 8.3%, as well as AV was common, caused mainly by *Staphylococcus aureus*, *E. coli*, and *Staph. Coagulase negative* 7.4%, 6.5% and 4.6% respectively. Then *Strep. pyogenes* and *Klebsiella spp* was 3.7% for each one. The results accepted with [20], reported *Candida* vaginitis was 20.4%, and *G.vaginalis* was 7.7% while *Staph spp.*, and *E. coli* were 9.9% and 2.4% respectively. *Streptococcus spp.*, and *Klebsiella spp.* were 6.1% and 1.5% respectively. Also, in the same line with [17, 21, 27], mentioned *C.albicans* was 11.9%, while *Staph. aureus* and Beta haemolytic *streptococci* were 7.4% and 3.6% respectively [21], (table 3).

The present study showed *G. vaginalis* was (100%) resistance to metronidazole. These agreed with [28], although the treatment of BV involve antibiotic and antimicrobials with oral or intravaginal metronidazole or clindamycin are associated with fairly good short-term cure rates, they fail to prevent BV in at least half of the cases in the long run [29].

The use of antiseptics and disinfectants for the treatment of vaginitis has been poorly studied and there is insufficient evidence at present to advocate the use of these agents. Antiseptic as medication, only mentioned by older and sporadic studies including chlorhexidin, povidin iodine or choramine as a preventive measure to prevent perinatal and maternal infectious complications in pregnancy [30].

A wide variety of natural products has been used in both disease prevention and treatment. In this study Alum (Aluminum potassium sulfate), a naturally occurring was tested as inhibitor bacterial and fungal growth. In addition, we used Gentian violet, which was introduced as an antiseptic by Sterling in 1890 and is used at a concentration of 1-2% in aqueous solutions [31]. Gentian violet is used in medicine as antibacterial, antifungal, and antiparasitic, it is a cationic triphenylmethane dye [32].

In the present study, we used Alum and Gentian violet in different concentrations (1% to 4%) against *G. vaginalis* and *C. albicans*. We found that, Alum inhibits growth of *G. vaginalis* and *C.albicans*. There is a significant correlation between the concentration of Alum and the Inhibition zone (mm) of *G. vaginalis* and *C.albicans* when the concentration increased the inhibition zone increased as shown in (table 5).

Also, Gentian violet inhibits growth of *G. vaginalis* and *C. albicans*, and there is a significant correlation between the concentration of Gentian violet and the Inhibition zone (mm) of *G. vaginalis* and *C. albicans*, when the concentration increased the inhibition zone increased as shown in (table 6).

Alum hydrolyzes in water to form sulfuric acid, which is responsible for rising the acidity in the environment therefore precipitate the protein [33], so the flagella formation and growth rate of bacteria will inhibition [34]. From the results alum had more effect against *G. vaginalis* isolates comparison with *C. albicans*, this effect may return to the efficiency of alum by change the pH of media. Alum effectively reduces bacteria because is an acidic producing compound that inhibits ammonia production by lowering pH, Hydrogen ions produced from the dissolution of alum react with ammonia to form nonvolatile ammonium($NH_4^+$) [35]. Alum reduces ammonia emissions by biological (inhibition of ureolytic microorganisms) and chemical means (conversion of $NH_3$ to $NH_4$–N). This

explain why alum electively reduces ammonia emissions. In a low-pH, environment where *G. vaginalis* and other microorganisms are inhibited. This result was agree with study of [36, 37, 38]. Alum has a low toxicity in experimental animals, and because the body does not absorb aluminum, it is harmless when swallowed, but ingestion of 30 grams (one ounce) has killed adult humans. Concentrated solutions have caused breakdown of gum tissues, kidney damage, and fatal intestinal bleeding [39]. Oneda and others proved that administration of Aluminum potassium sulphate (alum) 1.0, 2.5, 5.0 and 10.0% (w/w) does not exert tumorigenic or any other toxic actions in B6C3F1 mice [40]. The emergence of bacterial strains that exhibit resistance to various antibiotics and antimicrobials such as metronidazole, possess a major threat to medicine and public health. Consequently, there is renewed interest in antibacterial and antifungal targets, which by attenuating virulence disrupt the capacity of pathogenic bacteria to cause infection. Cheong and others proved that the combinations of aluminum with chlorhexidine or erythromycin are potentially useful as antibacterial agent. It is therefore, more beneficial to develop antibacterial agent using aluminum salts [41].

Gentian violet inhibits DNA replication in a number of bacteria and fungus [42] and several hypotheses have been provided to explain the selective toxicity of gentian violet in bacteria and trypanosomes [43], including alteration of the redox potential by the dye, inhibition of protein synthesis and disruption of $Ca^{+2}$ homeostasis. Gentian violet has been shown to depress protein synthesis in fibroblasts *in vitro* [44] and Hoffmann and co-workers [45] found that gentian violet is a potent inhibitor of amino acid transport and that this inhibition is apparently responsible for its inhibitory effect on *T. cruzi* protein synthesis. Gentian violet did induce an immediate increase in intracellular calcium concentrations and a large decrease in sodium levels suggesting the integrity of cellular membranes may have been compromised in influenza virus [46].

## Conclusion

Many genital infections occur because of new sexual practices, involving significant microbiological aspects. This could explain the presence new etiological agents of vaginal infections. *G.vaginalis* and *Candida spp.*, were the most causative agents of vaginitis. The Gram staining could be used to identify the pathogenic organisms involved in BV especially *G. vaginalis* (clue cells) since Gram staining is faster method, easy, and inexpensive, so that it could be used in developing countries, where the molecular techniques are not available. Also we suggest the use of metronidazole not always useful especially in those with recurrent vaginitis because of exaggerated use of its in Iraq even without examination & investigation to confirm the diagnosis. The alum and gentian violet stocks solutions at concentrations of (1% to 4%) were very effective against pathogenic bacteria and yeast vaginal infection compared with antimicrobials and antibiotics as (metronidazole, doxycycline and penicillin G).

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**Table 1: Distribution of vaginitis in married women.**

| Disease | Patient No. | % | Control No. | % |
|---|---|---|---|---|
| Yeast vaginitis | 100 | 46.51 | 0 | 0 |
| AV | 84 | 39.06 | 0 | 0 |
| BV | 18 | 8.37 | 0 | 0 |
| Other etiology | 13 | 6.04 | 0 | 0 |
| Total NO. | 215 | 100 | 65 | 100 |

**$X^2$=280, df= 4, P value < 0.05 significant**

**Table 2: Prevalence of obstetrical problems in patients with vaginitis.**

| Obst. Problems | Patients =215 | % | Controls =65 | % |
|---|---|---|---|---|
| Abortion 1st trimester | 40 | 18.6 | 8 | 12.3 |
| Abortion 2nd trimester | 14 | 6.5 | 1 | 1.5 |
| Abortion 3rd trimester | 18 | 8.4 | 2 | 3.1 |

**$X^2$=1.2 df =2, P value > 0.05 not significant**

**Table 3: Frequency of Microorganisms isolated from women with vaginitis.**

| Microorganism | Patient =215 | | Control =65 | | Chi, df, p value |
|---|---|---|---|---|---|
| | NO. | % | NO. | % | |
| *Candida albicans* | 70 | 32.6 | 0 | 0 | 28.217,1, <0.001 S |
| *Candida glabrata* | 10 | 4.7 | 0 | 0 | 3.135,1, 0.07 NS |
| *Sachrmycis cervisia* | 20 | 9.3 | 0 | 0 | 6.512,1,0.01 S |
| *Gardnerella vaginalis* | 18 | 8.4 | 2 | 3.07 | 2.11,1,0.14 NS |
| *Escherichia. Coli* | 25 | 11.6 | 1 | 1.53 | 6.03,1,0.01S |
| *Staphylococcus aureus* | 16 | 7.4 | 0 | 0 | 5.13,1,0.02S |
| *Staph.Coagulase negative* | 15 | 7.0 | 4 | 6.15 | 0.05,1,0.8 NS |
| *Streptococcus pyogenes* | 8 | 3.7 | 0 | 0 | 2.49,1,0.1 NS |
| *Enterococcus faecalis* | 6 | 2.8 | 5 | 7.69 | 3.18,1,0.07 NS |
| *Streptococcus agalactiae* | 6 | 2.8 | 14 | 21.53 | 26.4,1, <0.001 S |
| *Klebsiella spp.* | 8 | 3.7 | 0 | 0 | 2.49,1,0.1 NS |
| *Enterobacter spp.* | 6 | 2.8 | 0 | 0 | 1.85,1,0.2 NS |
| *Proteus spp.* | 3 | 1.4 | 0 | 0 | 0.9,1,0.3 NS |
| *Pseudomonas spp.* | 1 | 0.5 | 0 | 0 | 0.3,1,0.6 NS |
| *Enterococcus faecium* | 1 | 0.5 | 2 | 3.07 | 3.2,1,0.07 NS |
| *Enterococcus avium* | 2 | 0.9 | 0 | 0 | 0.9,1,0.3 NS |
| *Aer. Viridans* | 2 | 0.9 | 0 | 0 | 0.9,1,0.3 NS |
| *Streptococcus salivaris* | 1 | 0.5 | 0 | 0 | 0.3,1,0.6 NS |
| *NO growth of M.O.* | 13 | 6.0 | 37 | 56.92 | 88.1,1, <0.001 S |
| *Total* | 231 | | 65 | 100 | |

**Table 4: The antibiotics & antimicrobials used in this study.**

| Antibiotics & Antimicrobials | Symbol | Conc. Of antibiotics & antimicrobials (µg/disc) | S* | % | R* | % |
|---|---|---|---|---|---|---|
| Penicillin G | P | 10 | 3 | 16.7 | 15 | 83.3 |
| Cefotaxime | CFM | 5 | 10 | 55.6 | 8 | 44.4 |
| Doxycycline | DO | 30 | 4 | 22.2 | 14 | 77.8 |
| Metronidazole | MET | 30 | 0 | 0.0 | 18 | 100 |

S*: Sensitive, R*: Resistant

**Table 5: The univariate correlation between different Alum concentrations on *Candida albicans* and *Gardnerella vaginalis***

| | | Alum concentration (gm/dl) | Inhibition zone (mm) of *G. vaginalis* | Inhibition zone (mm) of *C. albicans* |
|---|---|---|---|---|
| Alum concentration (gm/dl) | PearsonCorrelation | 1 | 0.964** | 0.821* |
| | Sig. (2-tailed) | | 0.002 | 0.045 |
| | N | 6 | 6 | 6 |
| **. Correlation is significant at the 0.01 level (2-tailed). | | | | |
| *. Correlation is significant at the 0.05 level (2-tailed). | | | | |

**Table 6: The correlation between different concentrations of Gentian violet on *Candida albicans* and *Gardnerella vaginalis***

| | | Gentian violet concentration (gm/dl) | Inhibition zone (mm) of *G. vaginalis* | Inhibition zone (mm) of *C. albicans* |
|---|---|---|---|---|
| G.V concentration (gm/dl) | Pearson Correlation | 1 | 0.909* | 0.898* |
| | Sig. (2-tailed) | | 0.012 | 0.015 |
| | N | 6 | 6 | 6 |
| *. Correlation is significant at the 0.05 level (2-tailed). | | | | |

**Table 7: Effect of Alum & Gentian violet on *C.albicans* & *G.vaginalis***

| Solution type | Stock solution conc. % (gm/100ml) | Inhibition zone (mm) | |
|---|---|---|---|
| | | *C. albicans* | *G. vaginalis* |
| Alum | 0.1 | - | - |
| | 0.5 | - | 10 |
| | 1 | 10 | 12 |
| | 2 | 12 | 14 |
| | 3 | 13 | 16 |
| | 4 | 16 | 20 |
| Gentian violet | | | |
| | 0.1 | - | - |
| | 0.5 | 16 | 8 |
| | 1 | 18 | 10 |
| | 2 | 20 | 12 |
| | 3 | 21 | 15 |
| | 4 | 22 | 17 |