Impact of *Ferula hermonis* Roots Methanol Extract on Genotoxic, Biochemical and Reproductive Aspects of *Biomphalaria alexandrina* Snails

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**ABSTRACT**

One of the most significant neglected tropical diseases that have a detrimental effect on economic and public health criteria is Schistosomiasis. Therefore, there is a permanent and urgent need to find alternative means of biological control. *Ferula hermonis* is a herbaceous plant with various pharmaceutical properties and is known for its endocrine-disrupting effects. The objective of the present study was to investigate the molluscicidal activity of *F. hermonis* roots methanol extract against *Biomphalaria alexandrina* snails. The experimental results showed that *F. hermonis* extract has a molluscicidal activity against adult *B. alexandrina* snails at LC$_{50}$ (14.2 mg/l) and LC$_{90}$ (18.1 mg/l). Exposure of snails to sublethal concentrations LC$_5$ (8.3 mg/l) and LC$_{10}$ (9.8 mg/l) of the extract considerably lowered snail fertility and egg-laying capacity. In addition, results detected an endocrine-disrupting influence where it caused fluctuations in the levels of the three steroid sex hormones (Testosterone, Estradiol (E2), and Estrogen) following acute and chronic exposure of adult *B. alexandrina* snails to the sublethal doses of the tested extract. Regarding biochemical alterations, glucose and total lipids levels increased significantly, while protein and albumin contents decreased following exposure to sublethal concentrations, compared to the control snails. The sublethal concentrations (LC$_5$, LC$_{10}$, and LC$_{50}$) of *F. hermonis* roots methanol extract exerted noticeable DNA damage in *B. alexandrina* snails, whereas LC$_{10}$ was the most genotoxic concentration with 18.20±0.09% tailed cells and 10.54±0.58 px tail length. *F. hermonis* roots methanol extract can be considered a promising biocontrol agent against *B. alexandrina* snails, the intermediate host of *Schistosoma mansoni* in Egypt, via interference with the reproduction processes, and subsequently reducing the population size of snails in endemic areas.

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**INTRODUCTION**

Schistosomiasis is an acute and chronic parasitic disease caused by blood flukes (trematode worms) of the genus *Schistosoma*. More than 251.4 million people in 78 countries around the world are affected by this disease and hence require preventive treatment ([WHO, 2022](#)).

Snail control is considered one of the most important elements in the integrated strategy for the eradication of schistosomiasis ([Sokolow *et al.*, 2016](#)). The potential use of the molluscicide Niclosamide (Baylucide, Bayer®) is recommended by WHO in areas with a high frequency of schistosomiasis to control the mollusk population. However, as
a result of its high toxicity to non-target species such as fish, plants and other organisms in the aquatic ecosystem, novel methods of intermediate host control are required to be investigated (WHO, 2017; Silva et al., 2018).

Concerning the current situation in Egypt, active infections are still being recorded; according to a recent schistosomiasis study, (Haggag et al., 2017) reported that S. mansoni infection is still prevalent in various hotspot locations in Kafr El-Sheikh governorate, Egypt. Compared to summer, the autumn has a higher prevalence of human infection. However, most cases are of light intensity. Persistent transmission is associated with sociodemographic factors such as young age (6 –10 years), male sex and low maternal education level (Ghazy et al., 2022).

Regulation of the snail intermediate host’s fertility and egg-laying capacity is one of the proposed strategies to control the spread of the disease because it is obvious that Schistosomiasis infection rates and snail population density in endemic areas are directly correlated (Abu El Enin et al., 2019).

Ferula hermonis, also known as Shirsh-el-Zallouh, is a herbaceous plant from the Apiaceae family that is widely distributed throughout the Middle East. It has a long history of use in traditional medicine for the treatment of menopausal symptoms in women, erectile dysfunction in men, skin infections, stomach problems, fever, dysentery, and neurological conditions such as hysterias (Gamal-Eldeen & Hegazy, 2010; Hammam et al., 2022). The various bioactive ingredients that are primarily derived from F. hermonis’ roots, leaves and rhizomes are involved in its medicinal benefits (Maiuolo et al., 2022).

Sesquiterpene esters (ferritin, tenufertidene and ferritinol), which are recognized for their estrogenic properties, are among the numerous compounds found in F. hermonis (Sattar & Iranshahi, 2017). In vitro and in vivo pharmacological and biological properties of F. hermonis root extracts are referred to its richness in minerals such as magnesium, selenium, zinc, and iron in addition to its ability to be anti-inflammatory, antidiabetic, cytotoxic, anti-microbial and anti-fungal, anti-cancer and as a sexual activity enhancer (Geroushi et al., 2011; Safi et al., 2020; Al-Mutary, 2023; Kavaz & Faraj, 2023).

Based on the foregoing, the current study was designed to find a new control route for schistosomiasis by testing the endocrine-disrupting effect of F. hermonis roots methanol extract on B. alexandrina snails since interference with the reproduction processes could affect the population size of snails which is directly correlated to schistosomiasis transmission.

MATERIALS AND METHODS

Snails

Biomphalaria alexandrina snails used in this study were collected from the River Nile and irrigation channels in Giza governorate, Egypt then maintained in Medical Malacology Department, Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Snails were kept in standard plastic aquaria using dechlorinated aerated tap water, pH: 7 ± 0.2 and temperature (25 ± 2°C). Snails were provided with oven-dried lettuce leaves (ad libitum) for feeding (Eveland & Haseeb, 2011).
Plant extraction

*Ferula hermonis* roots were obtained from a local market in Egypt. The roots were ground into a powder, and the methanol extract was then prepared by soaking 250g of dry powder plant in 1000ml of methanol at room temperature for a week with daily stirring, followed by filtration and re-extraction for four times. The extract was filtered through Whatman filter paper No. 1 and concentrated with a rotatory evaporator at (50± 2°C). The crude extract was then collected and stored in a dark place at room temperature for future investigations.

Experimental design

A stock solution of methanol extract (1000ppm) was prepared (W/V) using dechlorinated tap water. A series of concentrations was prepared from stock solution (10, 12.5, 15, 17.5 and 20mg/ l) to calculate LC$_{50}$ and LC$_{90}$ (WHO, 1965). Three replicates were used, each of 10 snails (6–8 mm)/L of each concentration. Exposure and recovery periods were 24h for each test; three replicates of control snails were maintained under the same experimental conditions. The effectiveness of the tested extract were expressed in terms of LC$_{50}$ and LC$_{90}$.

Fecundity determination of *B. alexandrina* snails

Ninety mature snails (>10mm shell diameter) originated from the field population were tested for egg deposition. Snails were divided into 3 groups (each with 30 snails for 3 replicates); the first two groups were exposed to LC$_{5}$ (8.3mg/l) and LC$_{10}$ (9.8mg/l), respectively, and the third group was the control. Transparent plastic sheets measuring 5x5 cm were put in each aquarium for oviposition. Egg masses were weekly collected for each aquarium and counted under a stereo microscope. Dead snails were daily removed, while Lx (survival rates as a proportion of the correct one), Mx (number of eggs/snail/week) and R0 (reproductive rate: ∑LxMx) were weekly recorded (Habib *et al.*, 2020).

Estimation of steroid sex hormones of *B. alexandrina* snails exposed to *F. hermonis* roots methanol extract

The steroid hormones of testosterone, estradiol (E2) and estrogen were determined in the tissues of snails that survived post- continuous exposure to LC$_{5}$, LC$_{10}$ and LC$_{50}$ of the methanol extract for 3days (acute exposure) as well as in snails exposed to LC$_{5}$, and LC$_{10}$ for 4 successive weeks (chronic exposure) and in the control group. The steroid sex hormones were assessed using commercial enzyme immunoassay kits. The testosterone Kit was from AB Diagnostic Systems GmbH, Germany (cat. No. DK.040.01.3). The estradiol (E2) kit was from BioCheck, South San Francisco, CA 94080 (cat. No. BC-1111).The estrogen kit was obtained from Sun Red Biotechnology Company, Shanghai, China (cat. No. 201-02-0244). The absorbance of the calibrators, controls and test samples was measured with an immuno- spec microplate reader (Cat. No. INS28-CA-2000, Immuno-spec Corp., USA). The standard best-fit curve for each hormone was drawn through the plotted points following kit instructions.

Biomphalaria alexandrina snails' tissues preparation

Snails from treated and control groups were crushed between two slides, and the soft tissues were extracted and weighed then homogenized in phosphate buffer solution (PBS; 50 mM potassium phosphate, 1 mM EDTA, pH 7.5) in a 1:10 weight to volume ratio using a glass homogenizer. To investigate biochemical parameters and steroid sex
hormones, tissue homogenates were centrifuged at 950g for 10 minutes at 4°C. The supernatants were then kept at -80.

**Effect of methanol extract of *F. hermonis* on biochemical parameters of *B. alexandrina* snails**

Three groups of adult *B. alexandrina* snails were created. The first group (acute exposure) was continuously treated for three days with sublethal concentrations (LC₅, LC₁₀, and LC₅₀) of *F. hermonis* roots methanol extract. Another group (chronic exposure) was continuously exposed to LC₅ and LC₁₀ of the extract for 4 successive weeks, and the third was the control group.

Spectrophotometric measurements of biochemical parameters were made with the aid of kits from biodiagnostic company, Dokki, Giza, Egypt. Total protein, albumin, glucose and total lipids concentrations were estimated, respectively, according to the methods used in the studies of Domas (1975), Gustafsson (1976), Trinder (1969) and Knight *et al.* (1972).

**Determination of genotoxicity of *F. hermonis* roots methanol extract to *B. alexandrina* snails using comet assay**

*B. alexandrina* snails exposed to LC₅ and LC₁₀ of *F. hermonis* roots methanol extract for 4 weeks (chronic exposure) and in the control group were crushed, and the soft parts of snails were dissected out and kept in one ml of PBS at -4°C for further analysis. The tissues were then weighed and homogenized in an ice-cold phosphate buffer at a ratio of 1:10 W/V, using a glass homogenizer for 5 minutes. The homogenates were centrifuged for 15 minutes at 1,700g for 15min at 4°C. This suspension was stirred for 5min and filtered. Cell suspension (100 µl) was mixed with 600µl of low-melting agarose (0.8% in PBS). Then, 100µl of this mixture was spread on pre-coated slides. The coated slides were immersed in lysis buffer (0.045 M TBE, pH 8.4, containing 2.5% SDS) for 15min. The slides were placed in an electrophoresis chamber containing the same TBE buffer, but devoid of SDS. The slides were independently coded and scored.

The electrophoresis conditions were 2V/cm for 2min and 100mA. They were stained with ethidium bromide (20 µg/ml) at 4°C. The samples were investigated when they were still humid, and the DNA fragment migration patterns of 100 cells for each concentration level were evaluated with a fluorescence microscope (with an excitation filter of 420-490 nm [issue 510 nm]). Lengths of comet tails were measured from the middle of the nucleus to the end of the tail, and the size of the comet was determined. For visualization of DNA damage, observations were made of EtBr-stained DNA using a 40x objective on a fluorescent microscope. A comet 5 image analysis software developed by Kinetic Imaging, Ltd. (Liverpool, 198 UK) linked to a CCD camera was used to assess the quantitative and qualitative extent of DNA damage in the cells. It was carried out by measuring the length of DNA migration and the percentage of migrated DNA. Finally, the program calculated the tail moment. Generally, 50 to 100 randomly selected cells were analyzed per sample. % DNA in tail = total intensity of tail /total intensity of comet (head and tail) X100, and tail moment = tail length X % DNA in tail. Both the % of DNA in the tail and the tail moment are directly proportional to DNA damage (*Singh et al.*, 1988).
**Statistical analysis**

The probit facility of **Finney (1971)** was used to analyze the median values of lethal concentrations, while the Student's t-test was used to compare the mean values of the experimental and control groups (**Murray, 1981**). The statistical program SPSS version 20 for Windows (SPSS, Inc., Chicago, IL) was used to analyze the data. Values were expressed as mean ± standard error (S.E.).

**RESULTS**

**Ferula hermonis toxicity**

As shown in Table (1) and Fig. (1), the molluscicidal activity of the *F. hermonis* roots methanol extract to adult *B. alexandrina* snails was remarkable at the concentrations of LC$_{10}$ 9.8 mg/l and LC$_{50}$ 14.2 mg/l.

**Table 1.** Molluscicidal activity of *F. hermonis* roots methanol extract against adult *Biomphalaria alexandrina* snails after 24h of exposure

<table>
<thead>
<tr>
<th>LC$_5$</th>
<th>LC$_{10}$</th>
<th>LC$_{25}$</th>
<th>LC$_{50}$</th>
<th>LC$_{90}$</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td>8.3</td>
<td>9.8</td>
<td>11.4</td>
<td>14.2</td>
<td>18.1</td>
<td>1.25</td>
</tr>
</tbody>
</table>

**Fig. 1.** Concentration-mortality relationship (Logit transformed responses) of *F. hermonis* roots methanol extract against adult *B. alexandrina* snails after 24h of exposure
Effect of methanol extract of *F. hermonis* on survival rate (Lx), fecundity (Mx) and reproductive rate (R0) of adult *B. alexandrina* snails

The survival rate of snails exposed to LC5 and LC10 for 24 hours each week post four consecutive weeks were decreased to 0.6 and 0.7, respectively, compared to 0.95 for the control (Fig. 2A). Exposure to LC5 or LC10 significantly reduced the fertility of *B. alexandrina* snails (Fig 2B). After being exposed to LC10 of *F. hermonis* roots extract for 4 weeks, snails produced significantly fewer eggs per snail per week (P< 0.001).

For reproductive rate (R0), the present results elucidated that exposure of snails to LC5 and LC10 for 24hrs weekly of the tested extract for 4 successive weeks reduced reproduction of adult *B. alexandrina* snails by 57.37% and 60.41%, respectively (Fig. 2C).

Effect of *F. hermonis* roots methanol extract on the activity of steroid sex hormones

Exposure of *B. alexandrina* to sub-lethal concentrations of *F. hermonis* roots methanol extract had obvious effects on the activities of the three steroid sex hormones investigated in the tissues of snails (Fig 3). Acute exposure to LC5 and LC10 for 3 days led to an increase in testosterone levels, recording values of 7.1± 0.30 and 7.4± 0.23nmol/l, respectively, compared to 6.77± 0.31 nmol/l for the control group. While, a significant dose-independent decline was detected in the levels of testosterone following chronic exposure, and the lowest score for testosterone activity was observed after exposure to LC5 for 4 weeks, with values of 3.05± 0.35 (P< 0.01) nmol/ l, compared to the control record.

On the contrary, the levels of estradiol (E2) were not significantly affected with acute exposure, whereas chronic exposure to LC5 and LC10 for 4 weeks showed a remarkable elevation, exhibiting 41.5± 0.91, and 45.1± 0.98pg/ ml, respectively, compared to 36.9± 0.92pg/ ml for control group (Fig 3B).

The effect of sub-lethal concentrations of *F. hermonis* roots methanol extract on estrogen was fluctuating as acute exposure of snails to LC5, LC10, and LC50 reduced the hormone levels in a dose-dependent manner, recording 29.8±0.69 (P < 0.05) pg/ml after exposure to LC50 for 3 days compared to 34.1± 0.47pg/ml for the control group. On the other hand, chronic exposure to LC5 and LC10 for 4 weeks raised the levels of estrogen significantly to 49.9±0.82 and 41.2±1.35pg/ ml, respectively.
Fig. 2. (A) Survival rate (Lx), (B) Fecundity (Mx) and (C) Reproductive rate (Ro) of *B. alexandrina* snails exposed to LC5 and LC10 of methanol extract of *F. hermonis* (24h exposure weekly for 4 successive weeks).
Acute exposure of *B. alexandrina* snails to LC$_{50}$ of methanol extract of *F. hermonis* for 3 days led to a significant reduction in the glucose levels, recording values of 41± 1.5, compared to 71.3±2 mg/dl for the control group. On the other hand, chronic exposure for 4 weeks resulted in a significant dose-dependent elevation in glucose levels (Fig 4A).

Total lipids content was highly affected in *B. alexandrina* snails following acute exposure to *F. hermonis* methanol extract, recording the highest influence after exposure to LC$_{10}$ for 3 days being 200.7± 1.0, compared to 111.3± 0.2 mg/dl for the control snails. A similar pattern of elevation was noticed concerning both albumin and total protein contents in 3days of exposure, compared to the control snails, except LC50 that significantly reduced albumin level to 27.8g/ dL, compared to 35.13g/ dL for control group (Fig. 4C, D).
Fig. 4. Biochemical parameters in the tissue homogenate of *Biomphalaria alexandrina* snails, following exposure to methanol extract of *F. hermonis* for 3 days and 4 weeks

A: Glucose, B: Total lipids, C: Albumin and D: Total protein. Data were presented as Mean ± Standard Error. * Significance compared to control at $P<0.05$, ** at $P<0.01$ and *** at $P<0.001$

**Genotoxicity of *F. hermonis* roots methanol extract to *B. alexandrina* snails by comet assay**

Results in Table (2) and Fig (5) show the effect of sublethal concentrations (LC$_5$, LC$_{10}$, and LC$_{50}$) of *F. hermonis* methanol extract on the DNA of snails. All the tested concentrations resulted in noticeable DNA damage, compared to the control group, and LC$_{10}$ was the most genotoxic one with 18.20±0.09% tailed cells and 10.54±0.58 px tail length. The highest significant ($P<0.05$) tail moment was recorded in the DNA of snails exposed to LC$_{50}$ of *F. hermonis* extract, exhibiting 1.24±0.10 unit in comparison with 0.63±0.03 unit for the control group.
Fig. 5. Comet assay records showing: (A) Control group; (B) adult B. alexandrina snails after exposure to LC$_{5}$ of F. hermonis methanol extract; (C) after exposure to LC$_{10}$ and (D) after exposure to LC$_{50}$ (24hrs exposure weekly for 4 successive weeks).
Table 2. DNA damage criteria in Biomphalaria alexandrina tissues treated with sublethal doses of *F. hermonis* methanol extract according to comet assay

<table>
<thead>
<tr>
<th></th>
<th>Tailed %</th>
<th>Tail length (px)</th>
<th>% DNA in tail</th>
<th>Tail Moment (Unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>8.27±0.08</td>
<td>8.16±0.06</td>
<td>10.19±0.82</td>
<td>0.63±0.03</td>
</tr>
<tr>
<td><strong>LC₅</strong></td>
<td>16.43±0.27**</td>
<td>7.67±0.53</td>
<td>8.64±0.52</td>
<td>0.59±0.08</td>
</tr>
<tr>
<td><strong>LC₁₀</strong></td>
<td>18.20±0.09*</td>
<td>10.54±0.58*</td>
<td>9.86±0.22</td>
<td>0.61±0.09</td>
</tr>
<tr>
<td><strong>LC₅₀</strong></td>
<td>15.63±0.13**</td>
<td>9.26±0.49</td>
<td>12.07±0.13*</td>
<td>1.24±0.10*</td>
</tr>
</tbody>
</table>

Data represented as mean ± SE. * significant at *P* < 0.05 and ** at *P* < 0.01.
1 PX = 0.24 µm.

**DISCUSSION**

One way to tackle the problem of schistosomiasis is to delink or disintegrate the life cycle of the parasite by combating their respective snail hosts. The objectives of the present work targeted the evaluation of steroid hormones and reproductive parameters in snails, which might be playing a role in determining peculiarities governing snail parasite interactions. In addition, the genotoxic effect of *F. hermonis* may, in turn, affect snail reproduction and hence the prevalence of these snails and consequently reduce transmission of the diseases.

The present results showed that the methanol extract of *F. hermonis* has a molluscicidal activity against adult *B. alexandrina* snails at LC₅₀ (14.2 mg/l). In a previous study of *Iqbal and Sinha (2011)*, the gastropod *Biomphalaria glabrata* was used as a model organism to evaluate the endocrine-disrupting effect of bisphenol A and phthalate and their effect on the oviposition capacity of *B. glabrata*.

Interference with the reproduction process could affect the population size of snails. There is a direct correlation between schistosomiasis infection rates and the population size of snails in endemic areas (*King et al., 2006; Elsa et al., 2020*).

In this study, *F. hermonis* caused a significant progressive decline in the level of testosterone following both acute and chronic exposure, especially after exposure to LC₁₀ for 4 weeks, exhibiting 3.05± 0.35nmol/l, compared to 6.77± 0.31nmol/l for the control group. In general, the administration of *F. hermonis* utilized a significant negative impact on fertility outcomes. The effect of *F. hermonis* extract on testosterone observed in this study is in line with that of *Zanoli et al. (2003)* and *Al-Salhie and Al-Hummod (2019)* who reported that, the subchronic administration resulted in a significant decrease in testosterone level and marked affecion in the copulatory performance.

The effect of *F. hermonis* on male fertility was previously investigated by *Khleifat et al. (2001)* and *Hammam et al. (2022)*. They reported a decrease in the ability of male mice to mate after exposure to the aqueous extract of *F. hermonis* for 6 weeks.

In this study, *F. hermonis* roots extract showed a significant increase in estrogen levels in a dose-dependent manner. This may be explained by the catabolism of testosterone to 17-β estradiol. In males, testosterone is the major source of plasma
estradiol, the main biologically active estrogen (Ayuob et al., 2014). It was also established earlier that F. hermonis extract contains compounds such as ferrtin, feroline and tenuferidine. These compounds have estrogenic activity and structures that resemble diethyl-stilbesterol, a synthetic estrogen, which can increase the synthesis and the release of testosterone, which, in turn, enhances dopamine release. Dopamine has facilitative effects on sexual motivation, copulatory proficiency and genital reflexes (Hull et al., 2004; Kleitz-Nelson et al., 2010). Thus, reduced testosterone secretion results in copulatory dysfunction. Reduction in testosterone that occurred during the chronic administration of F. hermonis could be attributed to the negative feedback inhibition on the hypothalamus-pituitary-gonadal axis as was justified in the studies of Dillingham et al. (2005) and Blair et al. (2015).

The present data revealed that acute exposure of adult B. alexandrina snails to LC50 of methanol extract of F. hermonis for 3 days led to a significant reduction in glucose levels. A similar effect was reported by Raafat and El-Lakany (2015). The sugar-lowering effect was also seen as a consequence of F. gummosa administration at the lower dose of oleo-resin (100 mg/kg), but not at the higher dose (400 mg/kg), indicating that the anti-hyperglycemic effect of the extract is dose-dependant (Jalili-Nik et al. 2019). The increase following chronic exposure may be due to the fact that snails can reprogram their glucose uptake and metabolism while undergoing metabolic stress (Kim et al., 2017).

Regarding the genotoxicity, the present data revealed that the sub-lethal concentrations (LC5, LC10, and LC50) of F. hermonis methanol extract had a noticeable devastating effect on the DNA of snails. Whereas, LC10 was the most genotoxic concentration with the highest number of tailed cells.

A quick and accurate method for finding DNA strand breakage is the comet test, which tracks the movement of DNA from immobilized individual cell nuclei (Bolognesi & Cirillo, 2014). Several aquatic organisms including snails were investigated using this technique (Sarkare et al., 2015; Ibrahim et al., 2018). This method, which requires a minimal number of cells and can identify damage at the single-cell level, is regarded as one of the most promising genotoxicity indicators (Tamimet et al., 2019).

**CONCLUSION**

The sublethal concentrations of Ferula hermonis methanol extract had a multidimensional effect on Biomphalaria alexandrina snails. In snails, acute and chronic exposure to F. hermonis roots methanol extract, egg-laying capacity, fertility and disturbance in biochemical parameters, such as glucose, total protein, Albumin and total lipids were reduced. Furthermore, it had an endocrine-disrupting and genotoxic effects on treated snails, as evidenced by an overall increase in steroid hormone levels and DNA damage. Consequently, F. hermonis roots methanol extract can be considered a good candidate as a novel biocontrol method for schistosomiasis since interference with the reproduction processes in B. alexandrina snails could affect the population size of snails, which is directly correlated to schistosomiasis transmission.
LIST OF ABBREVIATIONS

E2: Estradiol
EtBr: Ethidium bromide
LX: Survival rates as a proportion of the correct one.
MX: Number of eggs/snail/week
PBS: Phosphate buffer solution
Px: Pixel
R0: Reproductive rate
SDS: Sodium Dodecyl Sulfate
TBE: Tris/Borate/EDTA
W/V: Weight per Volume

REFERENCES


