INVESTIGATION OF TOTAL PHENOLIC CONTENTS, ANTIBACTERIAL, ANTIFUNGAL AND ANTHELMINTIC POTENTIALS OF CRUDE METHANOLIC EXTRACT, SUBSEQUENT FRACTIONS AND CRUDE SAPONINS OF NONEA MICRANtha BOISS. & REUT.

Muhammad Imran¹, Farhat Ullah¹, Abdul Sadiq¹, Muhammad Ayaz¹*, Sajjad Ahmad¹, Zul Kamal², Anwar Zeb¹

¹Department of Pharmacy, University of Malakand, Chakdara, Dir (L), Pakistan
²Department of Pharmacy, Shaheed Benazir Bhutto University, Sharegail, Dir (U), Pakistan

ayazuop@gmail.com

Abstract

Nonea micrantha Boiss. & Reut. (N. micrantha) crude methanolic extract (CME), subsequent fractions and crude saponins (CSP) were investigated for antibacterial, antifungal, anthelmintic potentials and total phenolic contents. Chloroform fraction (CHF) exhibited the highest antibacterial activity against Pseudomonas aeruginosa (P. aeruginosa), Salmonella typhi (S. typhi), Escherichia coli (E. coli) and Klebsiella pneumoniae (K. pneumoniae). Aqueous fraction (AQF) showed prominent activity against Staphylococcus aureus (S. aureus). In determination of minimum inhibitory concentrations (MICs), CHF was found most active against P. aeruginosa, S. typhi, E. coli and K. pneumonia. In fungicidal assay, CSP was found most active against Aspergillus flavus (A. flavus) and Aspergillus niger (A. niger). While CME and CHF showed the strongest action against Fusarium oxysporum (F. oxysporum) and Aspergillus fumigatus (A. fumigatus). In anthelmintic activity, CHF and ethyl acetate fraction (EAF) demonstrated the highest anthelmintic activity against Ascaridia galli and Pheretima posthuma. In determination of total phenolics assay, EAF and CHF exhibited high phenolic contents. The results reveal that the plant is rich with phenolic compounds and possess bioactive compounds with antibacterial, antifungal and anthelmintic properties. The plant samples may be further investigated for isolation and structural elucidation of safe and effective antimicrobials compounds.

Keywords: Antibacterial, antifungal, anthelmintic, total phenolic contents, N. micrantha
**Introduction**

Infectious diseases caused by bacteria, fungi, viruses and parasites pose a major threat to public health, despite the significant progress in human medicines. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance [1]. In addition to this problem, antimicrobials are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions [2]. However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antimicrobials. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immunocompromised, AIDS and cancer patients. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants [3-4].

On other hand, helminthiasis is rampant worldwide. But due to poor personal and environmental hygiene conditions, it is more common in the developing countries. In the human body, many helminthes are found in GIT, but some also live in tissues. They harm the host by depriving him of food, thus leading to blood loss, injury to organs, intestinal or lymphatic obstruction and secretion of toxins. Though helminthiasis is rarely fatal, but it causes an ill health condition in a large portion of world population [5].

Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [6].

**N. micrantha** is species of family boraginaceae. Traditionally various species of boraginaceae are used in skin diseases, sore throat, gummosis, toothache, hepatic pain, stomach complaints, inflammation, bellyache, as diuretics and against anemia [8-9]. The antioxidant activity of most of the species of family boraginaceae has been reported [10]. Polyphenolic compounds have also been isolated from various species of this family with strong antioxidant activity [11-12].

Considering the vast potentiality of plants as sources for anthelmintic and antimicrobial drugs with reference to antibacterial and antifungal agents, this investigation was undertaken to screen CME, its subsequent fractions and CSP of *N. micrantha* (boraginaceae) for total phenolic contents, antibacterial, antifungal and anthelmintic activities.

**Methods and materials**

**Plant collection**

*N. micrantha* whole plant was collected in May 2013 from the hills of Dir Lower, Khyber Pakhtunkhwa, Pakistan. The plant was identified by plant taxonomist, Dr. Ali Hazrat, Department of Botany, SBU, Dir Upper (KPK) and deposited with voucher number 1021MI/SBHU in herbarium of afore mentioned university.

**Extraction**

The plant material was washed carefully with tap water and dried under shade at room temperature for two weeks. The shade dried (3 kg) parts of the plant were grinded properly and soaked in 80% methanol with occasional shaking. After 15 days, the whole suspension was filtered through muslin cloth. Thereafter the filtrate was concentrated under reduced pressure at 40°C using rotary evaporator (Heidolph, Germany). A residue of deep green color CME weighing 160g with a percent yield of 5.33% was obtained.

**Fractionation**

A portion of CME (140 g) was suspended in 500mL of distilled water and was consequently partitioned with n-hexane (3 × 500mL), chloroform (3 × 500mL), ethyl acetate (3 × 500mL), using separating funnels. Finally fractions NHF 10 g (7.14%), CHF 13g (9.28%), EAF 9 g (6.42%) and AQF 16 g (11.4%) were obtained.

**Extraction of crude saponins**

Plant powder material (60 g) was taken in a conical flask. Added 100mL of 20% ethanol to it and was
heated for 4 hours at 55°C with constant shaking in a water bath. Then this mixture was filtered and added 200mL of 20% ethanol to it. The volume of the extracting liquid was reduced to 40mL with the help of water bath and was transferred to separating funnel. Then 20mL of diethyl ether was added with vigorous shaking until two layers were formed. The organic layer was discarded and 60mL of n-butanol was added to the aqueous fraction in a separating funnel. The combined aqueous butanol mixture was washed with 5% NaCl solution several times for removal of impurities. The solvents were evaporated with the help of water bath leaving 7 g of crude saponins (11.66%) [13].

**Determination of total phenolic contents**

For determination of total phenolic contents, one ml from each concentration of the plant samples (1mg/mL) was added to 9 ml distilled water followed by addition of 1mL FCR with vigorous shaking. After five minutes, 10 ml of 7% Na2CO3 solution was added to each test sample and mixed properly. Distilled water (25mL) was added to the mixture and analyzed after 90 minutes using spectrophotometer (Thermo electron corporation, USA) at 750 nm. Finally gallic acid (97.5% pure) standard curve was employed to quantify total phenolic contents and were expressed as mg equivalent of gallic acid [14].

**Bacterial and fungal strains**

Antibacterial activity of *N. micrantha* was carried out using various bacterial strains, *E. coli* (739), *Salmonella typhi* (locally identified), *Klebsiella pneumoniae* (700603) and *P. aeruginosa* (27853) and *S. aureus* (29213). The fungicidal activity was investigated using *A. fumigatus*, *A. niger*, *A. flavus* and *F. oxysporum*. All the microorganisms were provided by Pharmaceutical Microbiology Laboratory, Department of Pharmacy, University of Malakand, Dir (L), Pakistan.

**Preparation and standardization of inoculums**

A loop full bacterial culture was immersed in the distilled sterile water and suspension with cell density of 1x10^8 CFU/ml, was prepared using McFarland standard and was further diluted to a cell density of 1x10^6 CFU/ml using a UV visible spectrophotometer (Thermo electron corporation USA) at 625 nm. Nutrient broth base powder was used for culturing fungal strains. The media was prepared according to manufacturer’s specifications and was transferred to sterile flasks at 65°C under laminar flow hood. These flasks were inoculated with the test fungi and were incubated at 25°C for primary growth.

**Antibacterial activity**

For determination of bactericidal potentials of plant samples disc diffusion assay was followed. Plant samples were dissolved in DMSO in a concentration of 10mg/mL. Nutrient agar media was prepared and transferred aseptically to sterilized Petri dishes and allowed to solidify. Each bacterial suspension (1mL) was uniformly spread on solid growth medium in Petri dishes under laminar flowhood. Sterile paper disks, 6 mm in diameter, (Whatman International, CAT: 2017-006) were impregnated with 100μL of plant samples and placed on the surface of each agar plate. Plates were incubated for 24 h at 37°C. Antibacterial potentials of the plant samples were determined by measuring the diameter of inhibition zone in mm. Disc impregnated with DMSO served as negative controls and disks with ceftriaxone (Geltis, Shaigan Pharmaceuticals) served as a positive control. Experiment was performed in triplicate [15].

**Determination of minimum inhibitory concentrations (MICs)**

The MIC values of the respective plant samples were determined following recommended procedure (16). The respective sample solution (1mL) at concentration of 20mg/mL was added to 1mL of sterilized nutrient broth. Subsequently, 1mL from the first test tube was transferred to the second test tube containing 1mL of nutrient broth and then these processes were continued up to the seventh test tube. Thereafter, 1mL of each test bacterium (1.0 x 10^6 CFU) was inoculated into each test tube and mixed thoroughly. The test tubes were then incubated at 37°C for 24 h. The MIC was taken as the lowest concentration that prevented the growth of bacterial culture.

**Antifungal assay**

To carry out antifungal assay, various plant samples were prepared in different concentrations (0.625-10mg/mL) by dissolving in DMSO. For the antifungal assay, Muller Hinton agar was prepared according to the manufacturer’s specifications by dissolving and autoclaving the specified quantity of the dry powder in sufficient quantity of distilled water and was transferred to sterile test tubes (10mL). To each test tube, 1mL solution of the test sample was added and
was inoculated with different fungal species. Test tubes were incubated at 25°C for 8 days and inhibition of fungal growth was observed. The MFC values were obtained in mg/mL. Nystatin (Nilstat, Pfizer Laboratories Ltd, Pakistan) was used as positive control. Experiment was performed in triplicate [17].

**Anthelmintic assays**

Adult roundworms (*Ascaridia galli*) and earthworms (*Pheretima posthuma* L. Vaill) were used to evaluate in vitro anthelmintic activity. The earthworms were collected from moist soil near University of Malakand, Dir, KPK, Pakistan. Roundworms were obtained from the intestines of freshly slaughtered domestic fowls. Their intestines were treated with normal saline solution to remove all the fecal matters. The worms were collected after dissection of intestines and maintained in normal saline solution, with an average size of roundworms and earthworms as 5-7 and 7-8 cm, respectively. The adult earthworms were used in the assay because of their high resemblance, both anatomically and physiologically, with the intestinal roundworm parasite *Ascaris lumbricoides* of human beings. The plant samples were prepared in distilled water at concentrations of 10, 20 and 40mg/ml. Six worms, approximately of equal size, each of *Pheretima posthuma* and *Ascaridia galli*, were placed in petri dishes. Each petri dish contained 25ml of test solution of plant samples. For reference standards, Albendazole and Piperazine citrate (10mg/ml each) were used as positive controls, and distilled water was used as the negative control. The experiments were performed in triplicate. Time for paralysis was recorded when no movement was observed except when shaken vigorously, whereas time of death was recorded when the worms did not show any movement by vigorous shaking nor when dipped in warm water (50°C) [18]

**Statistical analysis**

All the experiments were performed in triplicate and results were presented as mean ± SEM. One way ANOVA followed Dunnett’s Multiple Comparison Test for the comparison of positive control and test groups. *P* values < 0.05 were considered statistically significant. GraphPad Prism was used to draw the graphs.

**Results**

**Total phenolic contents**

The extraction yield of phenolics (mg GAE/g of sample) in CME, its subsequent fractions and CSP of the plant are summarized in Figure 1. EAF and CHF exhibited high phenolic contents with 280.00 ± 3.78 and 270.33 ± 2.60 mg GAE/g as compared to other fractions. The concentration of phenolics among different fractions were in an ascending order of EAF > CHF > NHF > CME > AQF.

**Bactericidal action**

The plant CME, its fractions and CSP showed antibacterial effect against different pathogenic bacteria, measured as diameter of inhibitory zone. CHF, AQF and NHF respectively, scored inhibitory zones of 23.55 ± 0.40, 19.65 ± 0.52 and 17.40 ± 0.68 mm against *P. aeruginosa*. Against *S. aureus*, AQF, NHF and CME showed the strongest activity with 33.71 ± 0.64, 30.66 ± 0.52 and 28.43 ± 0.44 mm zone of inhibition respectively as shown in Figure 2. Among other fractions, CHF and NHF were most active against *E. coli* with 27.59 ± 0.38 and 24.55 ± 0.81 mm inhibitory zone respectively. While CHF also exhibited the strongest action against *S. typhi* and *K. pneumoniae* with 16.62 ± 0.77 and 24.46 ± 0.34 mm inhibitory zone respectively as compared to standard ceftriaxone with 34.45 ± 0.77 mm inhibitory zone.

**Minimum inhibitory concentration (MIC)**

MIC values for CME, its subsequent fractions and CSP of *N. micrantha* are given in Table 1. Against *P. aeruginosa*, most prominent results were shown by CHF, AQF and NHF with MIC values of 2.08 ± 0.41, 2.08 ± 0.41 and 2.91 ± 1.10 mg/mL respectively. AQF and NHF were most active against *S. aureus* with MIC values of 0.83 ± 0.20 and 1.45 ± 0.55mg/mL respectively. Against *E. coli* CHF and AQF were found with the strongest acidity with MICs 1.66 ± 0.41 and 2.500 ± 0.00 mg/mL respectively. Similarly, CHF and AQF exhibited 3.33 ± 0.83 and 1.25.00 ± 0.00mg/mL MIC values against *S. typhi* and *K. pneumoniae* respectively.

**Fungicidal effect**

Antifungal activity of the *N. micrantha* samples was determined against four types of fungal species. CHF and CME were most effective against *A. fumigatus* with 1.75 ± 0.28 and 2.5 ± 0.14 mg/mL MFC values respectively. CSP was found effective against *A. flavus* and CME.
against A. niger with MFC values of 2.00 ± 0.38 and 1.5 ± 0.14 mg/mL respectively. Against F. oxysporum, CME showed strongest activity with 1.5 ± 0.14 mg/mL MFC values followed by CHF with 2.00 ± 0.14 mg/mL as represented in Table 2.

**Anthelmintic assays**

The anthelmintic potentials of N. micrantha samples were investigated against Ascaridia galli and Pheretima posthuma. Among all fractions, CHF, CSP and EAF expressed the highest anthelmintic activity against Ascaridia galli showing 31.00±2.00, 36.66±1.45 and 37.33±2.33 minutes death time and 16.33±1.45, 19.00±1.7619 and 33±1.45 minutes for paralysis respectively at 40mg/ml concentration. Similarly EAF, CHF and CSP were quite active against Pheretima posthuma showing 35.50±1.60, 36.16±1.30 and 40.66±1.20 minutes as death time respectively at 40mg/ml compared with standards used. All other fractions showed anthelmintic activity in concentration dependent manner, as shown in Table 3.

**Discussion**

Infectious diseases have caused much deaths worldwide because of multiple drug resistance development due to the indiscriminate use of commercial antimicrobial drugs. In addition to this problem, antimicrobials are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. Given the alarming incidence of antimicrobial resistance in bacteria and fungi of medical importance, there is a constant need for new and effective therapeutic agents, especially from natural products [19-20].

Plants and plant products have been extensively used for years in daily life to treat different infectious diseases all over the world. There has been continuous search to screen various natural products for their antimicrobial potentials. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world [21]. It has been reported that approximately 20% of the plants are found in the world have been submitted to pharmacological or biological test, and a substantial number of new antimicrobial introduced on the market are obtained from natural or semi-synthetic resources [22]. Screening N. micrantha for its antimicrobial potentials, it was found that CHF, AQF and NHF were prominently active against P. aeruginosa while against S. aureus, AQF, NHF and CME showed the strongest activity. Likewise, CHF and NHF were most active against E. coli. CHF also exhibited the strongest action against S. typhi and P. pneumonia. In fungicidal assay, CHF, CME and CSP were found active against most of the fungal strains.

A lot medicinal plants have been used to treat parasitic infections in man and animals. Parasitic helminthes affect the humans and animals leading to considerable hardship and underdeveloped growth. Parasitic attack is caused by a number of species of stomach and intestinal worms. Chemical control coupled with proper management has been vital worm control strategy throughout the world. Though extensive use of synthetic chemicals in modern clinical practices all over the world is prevailing, the exploiting of medicinal plants as source of drugs is still under study. This study reveals that N. micrantha samples are quite effective against helminthes. Among all fractions, CHF, EAF and CSP showed the highest activity against both Ascaridia galli and Pheretima posthuma compared with standards used in the assays.

Plants contain variety of secondary metabolites which are responsible for most of the pharmacological and biological activities of the plants. In recent years, secondary plant metabolites, previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents. The plants containing phenolic compounds have been reported for antimicrobial activities [23]. Significant attention has also been focused on phenolic compounds for their antioxidant potentials. Phenolic compounds have conjugated ring and hydroxyl groups in their structure which make them to function as antioxidants by scavenging the free radicals that are involved in oxidative processes [24]. The CME and its subsequent of N. micrantha were also screened for total phenolic contents and showed good extraction yield of phenols. EAF and CHF showed the highest quantity of phenol as compared to other fractions.

**Conclusion**

From our investigation of screening CME, its subsequent fractions and SCP of N. micrantha for total phenolic contents, antimicrobial and anthelmintic activities, the results confirm the therapeutic potency of the plant in bacterial, fungal and helminthes infections. The study suggests that some of the plant extracts are rich with phenolic
compounds and possess bioactive compounds with antibacterial, antifungal and anthelmintic properties. The most active extracts can be subjected to isolation and structural elucidation of the therapeutic antimicrobials and undergo further phytochemical and pharmacological evaluation. Further investigations are going on in our laboratory to isolate and identify the active components of the plant, accounting for the observed effects.

Acknowledgement

The authors want to thank University of Malakand for providing laboratory facilities to conduct the research.

References


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Figure 1: Total phenolic contents of different plant samples. Data was presented as mean ± SEM (n=3).
Figure 2: Bactericidal effect of CME, its fractions and CSP of *N. micrantha*. Data was represented as mean ± SEM (n=3). Values significantly different as compared to positive control, *: P < 0.05, **: P < 0.01, ***: P < 0.001. (DIZ: Diameter of inhibitory zones in mm).

### Table 1: MIC values for CME its subsequent fractions and CSP of *N. micrantha* against different bacterial strains (mg/mL)

<table>
<thead>
<tr>
<th>Samples</th>
<th><em>P. aeruginosa</em></th>
<th><em>S. aureus</em></th>
<th><em>E. coli</em></th>
<th><em>S. typhi</em></th>
<th><em>K. pneumonia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>CME</td>
<td>4.16 ± 0.83</td>
<td>1.66 ± 0.41</td>
<td>6.66 ± 1.66</td>
<td>3.75 ± 1.25</td>
<td>5.00 ± 0.00</td>
</tr>
<tr>
<td>NHF</td>
<td>2.91 ± 1.10</td>
<td>1.45 ± 0.55</td>
<td>3.33 ± 0.83</td>
<td>--------</td>
<td>8.33 ± 1.66</td>
</tr>
<tr>
<td>CHF</td>
<td>2.08 ± 0.41</td>
<td>4.16 ± 0.833</td>
<td>1.66 ± 0.41</td>
<td>3.33 ± 0.83</td>
<td>2.91 ± 1.10</td>
</tr>
<tr>
<td>EAF</td>
<td>5.00 ± 0.00</td>
<td>2.08 ± 0.41</td>
<td>5.00 ± 0.00</td>
<td>5.00 ± 0.00</td>
<td>2.08 ± 0.41</td>
</tr>
<tr>
<td>AQF</td>
<td>2.08 ± 0.41</td>
<td>0.83 ± 0.20</td>
<td>2.5 ± 0.00</td>
<td>6.66 ± 1.66</td>
<td>1.25 ± 0.00</td>
</tr>
<tr>
<td>CSP</td>
<td>--------</td>
<td>6.66 ± 1.66</td>
<td>5.00 ± 0.00</td>
<td>--------</td>
<td>4.16 ± 0.83</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.83 ± 0.20</td>
<td>0.625 ± 0.00</td>
<td>1.04 ± 0.20</td>
<td>1.25 ± 0.62</td>
<td>0.625 ± 0.00</td>
</tr>
</tbody>
</table>

Data was represented as mean ± SEM (n=3)

### Table 2: Fungicidal action of CME, its subsequent fractions and CSP of *N. micrantha* against different fungal strains. (MFCs in mg/ml)

<table>
<thead>
<tr>
<th>Fungi</th>
<th>CME</th>
<th>NHF</th>
<th>CHF</th>
<th>EAF</th>
<th>AQF</th>
<th>CSP</th>
<th>Nystatin</th>
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</thead>
<tbody>
<tr>
<td><em>A. fumigatus</em></td>
<td>2.50 ± 0.14</td>
<td>4.75 ± 0.14</td>
<td>1.75 ± 0.28</td>
<td>2.75 ± 0.14</td>
<td>3.00 ± 0.28</td>
<td>6.83 ± 0.22</td>
<td>0.75 ± 0.14</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>2.50 ± 0.22</td>
<td>5.33 ± 0.22</td>
<td>2.25 ± 0.25</td>
<td>3.00 ± 0.22</td>
<td>5.50 ± 0.28</td>
<td>2.00 ± 0.38</td>
<td>0.66 ± 0.08</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>1.50 ± 0.14</td>
<td>2.75 ± 0.14</td>
<td>2.41 ± 0.22</td>
<td>3.00 ± 0.33</td>
<td>2.83 ± 0.22</td>
<td>1.75 ± 0.14</td>
<td>0.50 ± 0.14</td>
</tr>
<tr>
<td><em>F. oxysporum</em></td>
<td>1.50 ± 0.14</td>
<td>4.66 ± 0.84</td>
<td>2.00 ± 0.14</td>
<td>2.75 ± 0.14</td>
<td>5.91 ± 0.30</td>
<td>7.00 ± 0.38</td>
<td>0.75 ± 0.14</td>
</tr>
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</table>

Data was represented as mean ± SEM (n=3)
<table>
<thead>
<tr>
<th>Samples</th>
<th>Concentration (mg/ml)</th>
<th>Ascaridia galli</th>
<th>Pheretima posthuma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Paralysis Time (in minutes)</td>
<td>Death Time (in minutes)</td>
</tr>
<tr>
<td>CME</td>
<td>40</td>
<td>21.66 ± 1.20</td>
<td>42.00 ± 2.08</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>29.00 ± 0.57</td>
<td>48.33 ± 1.43</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>32.33 ± 1.76</td>
<td>59.66 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>34.00 ± 2.00</td>
<td>62.33 ± 2.60</td>
</tr>
<tr>
<td>NHF</td>
<td>20</td>
<td>39.00 ± 1.15</td>
<td>71.33 ± 2.33</td>
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<td>10</td>
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<td>80.00 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>16.33 ± 1.45</td>
<td>31.00 ± 2.00</td>
</tr>
<tr>
<td>CHF</td>
<td>20</td>
<td>21.00 ± 1.52</td>
<td>38.66 ± 2.72</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>29.00 ± 0.57</td>
<td>42.00 ± 1.52</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>19.33 ± 1.45</td>
<td>37.33 ± 2.33</td>
</tr>
<tr>
<td>EAF</td>
<td>20</td>
<td>29.66 ± 2.18</td>
<td>46.66 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>32.00 ± 1.52</td>
<td>54.00 ± 1.15</td>
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<td></td>
<td>40</td>
<td>24.66 ± 0.66</td>
<td>37.00 ± 0.57</td>
</tr>
<tr>
<td>AQF</td>
<td>20</td>
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<td>47.33 ± 1.20</td>
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<tr>
<td></td>
<td>10</td>
<td>38.33 ± 0.66</td>
<td>58.00 ± 1.73</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>19.00 ± 1.76</td>
<td>36.66 ± 1.45</td>
</tr>
<tr>
<td>CSP</td>
<td>20</td>
<td>28.00 ± 0.57</td>
<td>42.83 ± 1.36</td>
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<tr>
<td></td>
<td>10</td>
<td>39.33 ± 0.88</td>
<td>54.10 ± 0.95</td>
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<tr>
<td>Albendazole</td>
<td>10</td>
<td>14.00 ± 1.52</td>
<td>37.66 ± 0.88</td>
</tr>
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<td>Piperazine citrate</td>
<td>10</td>
<td>9.83 ± 0.72</td>
<td>21.33 ± 0.33</td>
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</tbody>
</table>

Data was represented as mean ± SEM (n=3)