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

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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]. The potential for developing antimicrobials from higher plants appears rewarding, as it will lead to the development of phyto-medicine to act against microbes. Plants based antimicrobial studies have enormous therapeutic potential as they are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [7].

N. micrantha is specie 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, SBBU, Dir Upper (KPK) and deposited with voucher number 1021MI/SBBU 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 40̊C 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 1×10^6 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 flaks 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 (1.0×10^6 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 earth worms 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 valves 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 2.5.00 ± 0.00 mg/mL respectively. Similarly, CHF and AQF exhibited 3.33 ± 0.83 and 1.25.00 ± 0.00mg/mL MIC valves 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. oxysporumm*, 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 world-wide 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.

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References

1. Okeke I N, Laxminarayan R, Bhutta Z A, et al. Antimicrobial resistance in developing countries. Part I: recent trends and current status. The Lancet infectious diseases 2005; 5: 481-493.
2. Ahmad I, Mehmood Z and Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. Journal of ethnopharmacology 1998; 62: 183-193.
3. Rinaldi M G. Problems in the diagnosis of invasive fungal diseases. Review of infectious diseases 1991; 13: 493-495.
4. Diamond R D. The growing problem of mycoses in patients infected with the human immunodeficiency virus. Review of infectious diseases 1991; 13: 480-486.
5. Panda S, Das D and Tripathy N. Evaluation of Anthelmintic activity of *Chlorophytum borivilianum* santapau & fernandes. International Journal of Research in Pharmaceutical and Biomedical Sciences 2011; 2: 676-679.
6. Rojas R, Bustamante B, Bauer J, et al. Antimicrobial activity of selected Peruvian medicinal plants. Journal of ethnopharmacology 2003; 88: 199-204.
7. Yasunaka K, Abe F, Nagayama A, et al. Antibacterial activity of crude extracts from Mexican medicinal plants and purified coumarins and xanthones. Journal of ethnopharmacology 2005; 97: 293-299.
8. Shah A, Marwat S K, Gohar F, et al. Ethnobotanical Study of Medicinal Plants of Semi-Tribal Area of Makerwal & Gulla Khel (Lying between Khyber Pakhtunkhwa and Punjab Provinces), Pakistan. American Journal of Plant Sciences 2013; 4.
9. Di Stasi L, Oliveira G, Carvalhaes M, et al. Medicinal plants popularly used in the Brazilian Tropical Atlantic Forest. Fitoterapia 2002; 73: 69-91.
10. Okusa P, Penge O, Devleeschouwer M and Duez P. Direct and indirect antimicrobial effects and antioxidant activity of Cordia gilletii De Wild (Boraginaceae). Journal of Ethnopharmacology 2007; 112: 476-481.
11. Kelley C J, Harruff R C and Carmack M. Polyphenolic acids of *Lithospermum ruderale*. II. Carbon-13 nuclear magnetic resonance of lithospermic and rosmarinic acids. The Journal of Organic Chemistry 1976; 41: 449-455.
12. Rice-evans C A, Miller N J, Bolwell P G, Bramley P M and Pridham J B. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free radical research 1995; 22: 375-383.
13. Khan F A, Ullah Z and Haider S. Phytochemicals screening and antimicrobial activities of selected medicinal plants of Khyberpakhtunkhwa Pakistan. African Journal of Pharmacy and Pharmacology 2011; 5: 746-750.
14. Ayaz M, Junaid M, Ahmed J, et al. Phenolic contents, antioxidant and anticholinesterase potentials of crude extract, subsequent fractions and crude saponins from *Polygonum hydropiper* L. BMC complementary and alternative medicine 2014; 14: 145.
15. Bauer A, Kirby W, Sherris J C, turck and Turck M. Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology 1966; 45: 493.
16. Akinpelu D and Kolawole D. Phytochemical and antimicrobial activity of leaf extract of *Piliostigma thonningii* (Schum.). Sci. Focus J 2004; 7: 64-70.
17. Shah S M, Ayaz M, Khan A-u, et al. 1, 1-Diphenyl, 2-picrylhydrazyl free radical scavenging, bactericidal, fungicidal and leishmanicidal properties of *Teucrium stocksianum*. Toxicology and industrial health 2013: 0748233713487250.
18. Parida S, Patro V J, Mishra U S, Mohapatra L and Sannigrahi S. Anthelmintic potential of crude extracts and its various frations of different parts of *Pterospermum acerifolium* Linn. Inter J Pharma Sci Rev Res 2010; 1: 107-11.
19. Davies J. Inactivation of antibiotics and the dissemination of resistance genes. Science 1994; 264: 375-382.
20. Monroe S and Polk R. Antimicrobial use and bacterial resistance. Current opinion in microbiology 2000; 3: 496-501.
21. Ates D A and Turgay Ö. Antimicrobial activities of various medicinal and commercial plant extracts. Turkish Journal of Biology 2009; 27: 157-162.
22. Mothana R A and Lindequist U. Antimicrobial activity of some medicinal plants of the island Soqotra. Journal of ethnopharmacology 2005; 96: 177-181.
23. Rauha J-P, Remes S, Heinonen M, et al. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. International journal of food microbiology 2000; 56: 3-12.
24. Shahidi F, Janitha P and Wanasundara P. Phenolic antioxidants. Critical Reviews in Food Science & Nutrition 1992; 32: 67-103.

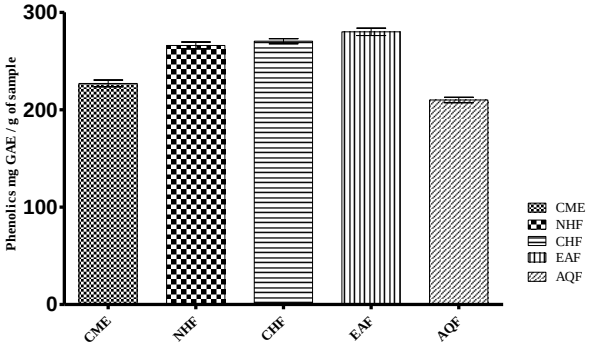
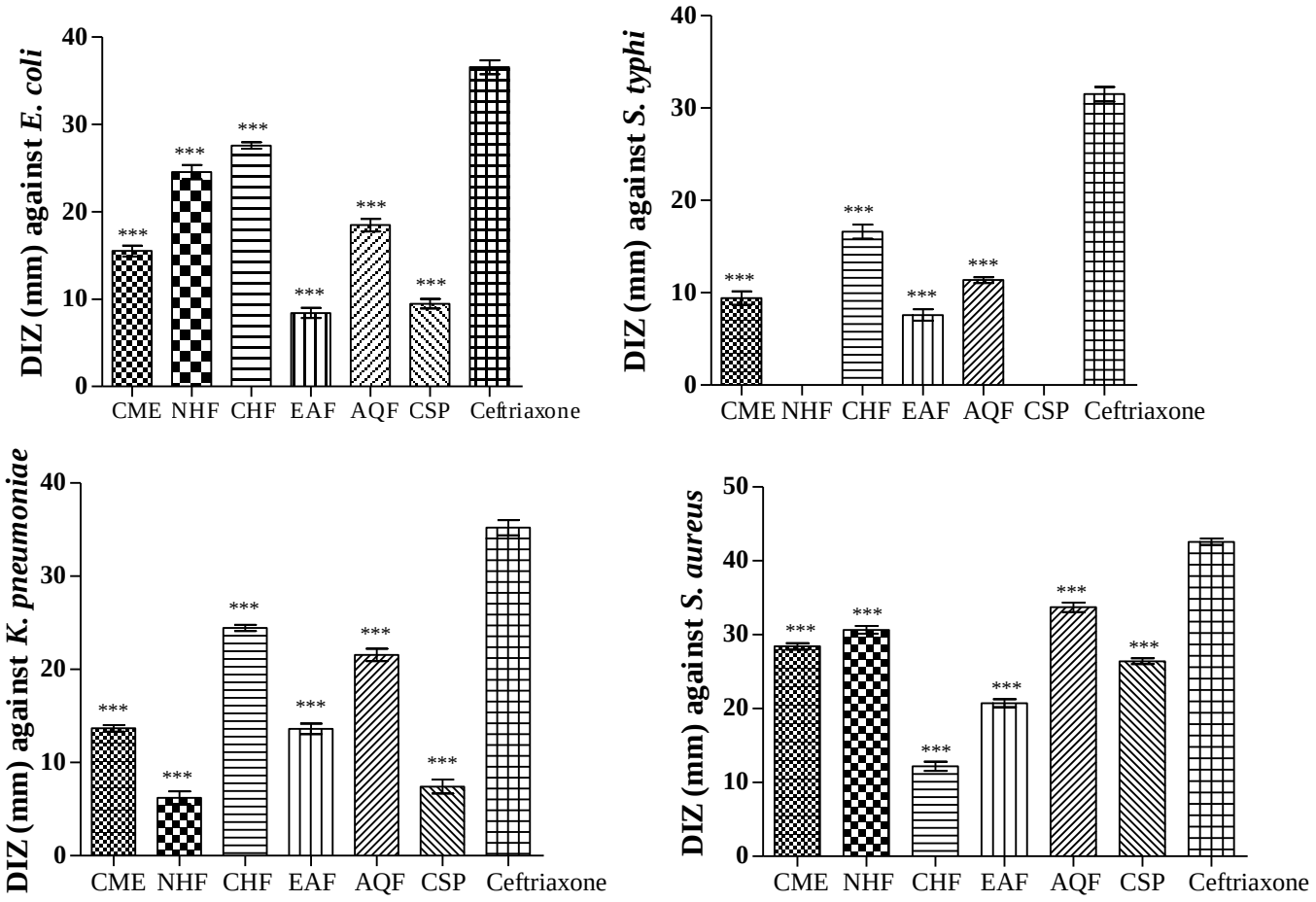


Figure 1: Total phenolic contents of different plant samples. Data was presented as mean ± SEM (n=3).



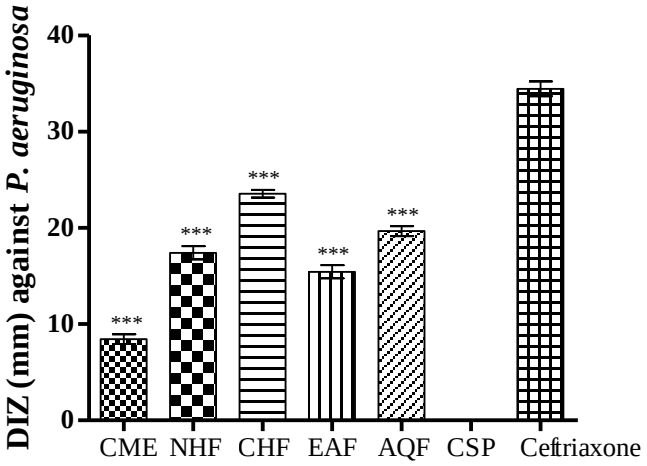


Figure 2: Bactericidal effect of CME, its fractions and CSP of *N. micrantha.* Data was represented as mean ± SEM (n**=3**). Values significantly different as compare to positive control, *:P < 0.05, ** :P < 0.01, ***: P < 0.001. (DIZ: Diameter of inhibitory zones in mm).

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

Samples	*P. aeruginosa*	*S. aureus*	*E. coli*	*S. typhi*	*K. pneumonia*
CME	4.16 ± 0.83	1.66 ± 0.41	6.66 ± 1.66	3.75 ± 1.25	5.00 ± 0.00
NHF	2.91 ± 1.10	1.45 ± 0.55	3.33 ± 0.83	--------	8.33 ± 1.66
CHF	2.08 ± 0.41	4.16 ± 0.833	1.66 ± 0.41	3.33 ± 0.83	2.91 ± 1.10
EAF	5.00 ± 0.00	2.08 ± 0.41	5.00 ± 0.00	5.00 ± 0.00	2.08 ± 0.41
AQF	2.08 ± 0.41	0.83 ± 0.20	2.5 ± 0.00	6.66 ± 1.66	1.25 ± 0.00
CSP	--------	6.66 ± 1.66	5.00 ± 0.00	---------	4.16 ± 0.83
Ceftriaxone	0.83 ± 0.20	0.625 ± 0.00	1.04 ± 0.20	1.25 ± 0.62	0.625 ± 0.00

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)

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

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

Table 3: Anthelmintic effects of CME, its subsequent fractions and CSP of *N.micrantha* against *Ascaridia galli* and *Pheretima posthuma*

Samples	Concentration (mg/ml)	*Ascaridia galli*		*Pheretima posthuma*	
		Paralysis Time (in minutes)	Death Time (in minutes)	Paralysis Time (in minutes)	Death Time (in minutes)
CME	40	21.66 ± 1.20	42.00 ± 2.08	23.66 ± 0.88	44.16 ± 1.16
	20	29.00 ± 0.57	48.33 ± 1.43	35.66 ± 0.66	61.00 ± 1.04
	10	32.33 ± 1.76	59.66 ± 0.88	39.00 ± 0.57	69.33 ± 0.88
NHF	40	34.00 ± 2.00	62.33 ± 2.60	32.00 ± 1.00	42.16 ± 0.44
	20	39.00 ± 1.15	71.33 ± 2.33	36.66 ± 1.45	51.33 ± 0.92
	10	45.33 ± 2.33	80.00 ± 0.57	38.16 ± 0.72	63.00 ± 1.73
CHF	40	16.33 ± 1.45	31.00 ± 2.00	16.00 ± 1.52	36.16 ± 1.30
	20	21.00 ± 1.52	38.66 ± 2.72	23.33 ± 1.33	42.33 ± 1.45
	10	29.00 ± 0.57	42.00 ± 1.52	27.66 ± 2.02	53.16 ± 2.16
EAF	40	19.33 ± 1.45	37.33 ± 2.33	21.00 ± 1.00	35.50 ± 1.60
	20	29.66 ± 2.18	46.66 ± 0.88	25.66 ± 0.88	39.83 ± 0.72
	10	32.00 ± 1.52	54.00 ± 1.15	33.88 ± 1.30	51.16 ± 1.01
AQF	40	24.66 ± 0.66	37.00 ± 0.57	23.00 ± 1.15	52.66 ± 1.20
	20	31.00 ± 1.00	47.33 ± 1.20	34.16 ± 1.74	61.83 ± 1.69
	10	38.33 ± 0.66	58.00 ± 1.73	38.66 ± 0.88	68.00 ± 1.15
CSP	40	19.00 ± 1.76	36.66 ± 1.45	21.66 ± 1.45	40.66 ± 1.20
	20	28.00 ± 0.57	42.83 ± 1.36	32.00 ± 1.00	55.83 ± 0.72
	10	39.33 ± 0.88	54.10 ± 0.95	40.16 ± 1.42	69.00 ± 1.73
Albendazole	10	14.00 ± 1.52	37.66 ± 0.88	17.33 ± 1.20	48.33 ± 1.45
Piperazine citrate	10	9.83 ± 0.72	21.33 ± 0.33	10.00 ± 0.28	29.66 ± 0.66
Negative control	-------	-------	-------	--------	---------

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