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Research Article

Phytochemical Investigation and Antimicrobial Potential of Medicinal Plant *Nepeta distans* Royle ex Benth

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Herbal medicines or natural products and plant extract may exhibit promising alternatives or supplements for chemotherapy and antibiotic therapy. The aim of the study is to evaluate the therapeutic value and phytoconstituents of the whole plant, *Nepeta distans*. The methanol extract contains four known compounds, namely, oleanolic acid, ursolic acid, β-sitosterol, and stigmasterol. The structures of these compounds were confirmed with the help of NMR and mass spectrometry and by comparison with the available literature of these known compounds. The phytochemical analysis test confirmed the presence of alkaloids, flavonoids, saponins, glycosides, fats, proteins, and phytosterols. The antimicrobial activities were carried out by the agar well diffusion method. Both methanol extract and chloroform fraction showed significant antimicrobial activities.

1. Introduction

Since time unknown, medicinal plants are used for the treatment of different infections. The World Health Organization reports that various plant fractions and their dynamic constituents are utilized as traditional medicines by 80% of the world population [1–4]. Herbal medicines or natural products and plants extract may exhibit promising alternatives or supplements for chemotherapy and antibiotic therapy [5, 6]. Herbal medicines have astonishing potential such as high anticancer, antibacterial, antioxidant, and antifungal activities [7, 8]. Plants offer an inexhaustible source of bioactive compounds and clinically useful drugs for infectious diseases, such as cancer and cardiovascular disorders [9–11]. Bioactive compounds play an important role in drug discovery [12]. However, the increasing antimicrobial resistance to the currently available antimicrobial agents demand intense investigations into the antimicrobial properties of the medicinal plants [13–15]. Regrettably, there has been limited pharmaceutical development of the plants with known bioactivity [16, 17]. There are few pharmaceutical products for infectious diseases that are of plant origin [18, 19]. To fulfill the healthcare needs in Germany and Europe, herbal preparation got more attention, and nearly 1400 herbal preparations are presently used as per European Union [20, 21]. The herbal preparation can also be utilized in various cosmetic industries (as antiwrinkling agents, skin tissue regenerators, and antiage creams) which can lead to an increase in the importance [22, 23].

The genus *Nepeta* (Lamiaceae) is a large family which comprises about 400 species, most of which grow in the wild in the central and southern parts of Europe, North Africa, and central and southern Asia. A lot of species of this genus are used in the folk medicine for the antiseptic and astringent properties as topical remedies in children's cutaneous eruptions and snakes and scorpion bites; orally, they are utilized as antitussive, antispasmodic, antiasthmatic, febrifuge, and diuretic. Moreover, antibacterial, fungicidal, and antiviral activities have been attributed to *Nepeta* lactones and iridoids contained in several *Nepeta* species. Some endemic species in the southern Greece are utilized in traditional medicine. In particular, fresh leaves of some *Nepeta* species are chewed to alleviate toothache and a leaf alcoholic macerate is efficacious for treatment of contusions and rheumatic pains [24]. This genus is also reported to possess biological activities that help in reduction of serum lipids and possess anti-inflammatory, phytotoxic, platelet aggregation, antimicrobial, cytotoxic, and antiglycation properties [25].

The aim of the present study is to screen out the bioactive fractions of *Nepeta distans*, for isolation of the targeted compounds in future.

2. Materials and Methods

2.1. Plant Collection. The plant *Nepeta distans* was collected from Darra Adam Khel district, Kohat (33.6945°N, 71.4959°E), Pakistan. The collected plants were then identified by a plant taxonomist at the Department of Chemical and Life Sciences, Qurtuba University of Science and Information Technology, Peshawar. It was then cleaned, washed, and dried in the shade. A specimen voucher number (ND-001) was deposited in the herbarium of the same department.

2.2. Extraction, Fractionation, and Isolation. The whole plant of *N. distans* was grinded to a coarse powder. The powdered plant (2 Kg) was initially extracted with 20 L of 70% MeOH three times at room temperature. It was then filtered and methanol was evaporated under reduced pressure leaving behind a greenish, syrup residue that was dried and weighed. It was 100 g. This MeOH extract was then partitioned into various fractions through a separating funnel. It was partitioned into hexane, chloroform, ethyl acetate, butanol, and water fractions successively. The methanol extract was then subjected to column chromatography which afforded compounds (1–4) from different subfractions at different polarities of the binary solvent system of chloroform/hexane and ethyl acetate/hexane.

2.3. Preliminary Phytochemical Qualitative Tests. Alkaloid, flavonoids, saponins, proteins, oils, and glycosides were tested in the crude extract of *Nepeta distans* using several qualitative tests [26–29].

2.4. Antimicrobial Activity. The antifungal and antimicrobial activities of the methanol extract and chloroform fraction of *N. distans* were conducted as per available method in the literature [30–34].

2.4.1. Antifungal Activity. Using the agar tube dilution method, the antifungal potential of the methanol extract and chloroform fraction of *Nepeta distans* was investigated. The stock solution was made with 24 mg extracts per ml (1.0 ml) of sterile DMSO (dimethyl sulfoxide). The Sabouraud dextrose agar medium was made by using a magnetic stirrer to thoroughly dissolve 4.0 g of agar, 4 percent of glucose agar, and 32.5 g of Sabouraud in distilled water (500 ml) to form a homogenous slurry. 4 ml of SDA (Sabouraud dextrose agar) was autoclaved at 120°C for 15 minutes before being chilled to 15°C. When each test tube was injected (4 mm diameter) with fungal strains of 6-7 days old culture for non-mycelia development, an agar surface was created by mixing the stock solution with the nonsolidified SDA medium, and solidifying it in a tilted position, streak was formed. Positive and negative controls were preserved for treatment evaluation by employing DMSO and antifungal medications, respectively (amphotericin B). Subsequent to 7 days of incubation at 27°C, the fungal growth reserve was measured.

2.4.2. Antibacterial Activity. Using the well diffusion agar method, the antibacterial activity of methyl alcohol and chloroform extracts of *Nepeta distans* was investigated. Bacterial strains were activated by infusing the nutrient broth into the conical flasks and rearing the medium for 24 hours. The agar media was then transferred to Petri dishes containing the injected bacterial strains and solidified under the appropriate conditions. After the medium had solidified, the bores were made in the agar plate using a corkborer, and the extracts (50 l) were transferred to the bore and the plates were reared for 24 hours at 37°C. The results were expressed as the bacterial growth inhibition zone in millimeters. Amoxicillin and ciprofloxacin were employed as positive controls for Gram-positive and Gram-negative bacteria, respectively, and DMSO was utilized as the negative control retain for the purpose of determining the effect.

3. Results and Discussion

3.1. Isolation of Phytoconstituents. The methanol extract of *N. distans* was subjected to column chromatography and the afforded four compounds were oleanolic acid [35], ursolic acid [36], β-sitosterol [37], and stigmasterol [37]. The structure of these compounds are given in Figure 1.

*3.1.1. Spectral Data of Compound **(1)**.* HR-EI-MS *m/z*: 456.2 $[M]^+$. ^{1}H-NMR ($CDCl_3$, 400 MHz) (δ ppm) 0.88 (3H, each *s*, Me), 3.61 (1H, dd, $J = 4.1$, 9.91 Hz, H-3), 5.24 (1H, *t*, $J = 3.45$ Hz, H-12), 1.12, 1.04, 0.98, 0.97, 0.91. ^{13}C-NMR ($CDCl_3$, 100 MHz) (δ ppm) 15.43 (*q*, C-25), 15.76 (*q*, C-24), 17.21 (*q*, C-26), 18.9 (*t*, C-6), 23.4 (*t*, C-16), 23.41 (*t*, C-11), 23.54 (*q*, C-30), 25.97 (*q*, C-27), 27.37 (*t*, C-2), 27.91 (*t*, C-15), 28.34 (*q*, C-23), 30.83 (*s*, C-20), 33.9 (*t*, C-21), 42.31 (*s*, C-14), 32.4 (*t*, C-22), 183.2 (*s*, C-28), 32.7 (*t*, C-7), 33.1 (*q*, C-29), 37.21 (*s*, C-10), 143.7 (*S*, C-13), 38.32 (*t*, C-1), 38.86 (*s*, C-4), 39.29 (*s*, C-8), 121.97 (*d*, C-12), 41.52 (*d*, C-18), 45.99 (*t*, C-19), 48.13 (*d*, C-9),55.43 (*d*, C-5), 46.47 (*s*, C-17), 79.12 (*d*, C-3).

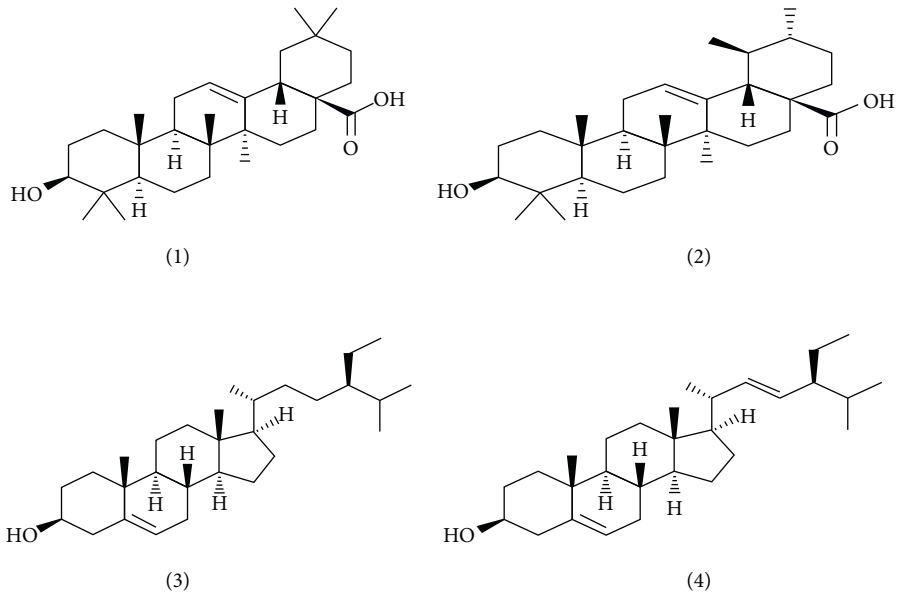

Figure 1: Structures of isolated compounds (1–4).

3.1.2. Spectral Data of Compound ***(2)****.* EI-MS *m/z* (rel. int. %): 456 $[M]^+$ (16), 55 (49), 119 (29), 248 (100), 300 (11), 203 (44), 207 (26), 133 (60), ^{1}H-NMR ($CDCl_3$, 400 MHz) (δ ppm) 0.80 (3H, $J = 6.8$ Hz, Me-29), 0.81 (3H, *s*, Me-24), 0.86 (3H, *s*, Me-26), 0.91 (3H, *d*, $J = 6.6$ Hz, Me-30), 5.11 (1H, *m*, H-12), 1.07 (3H, *s*, Me-23), 0.94 (3H, *s*, Me-25), 3.19 (1H, dd, $J_{ax,\ ax} = 10.0$ Hz, $J_{ax,\ eq} = 4.5$ Hz, H-3α), 1.20 (3H, *s*, Me-27). ^{13}C-NMR ($CDCl_3$, 125 MHz) (δ ppm) 15.4 (*q*, C-24), 138.7 (*s*, C-13), 15.9 (*q*, C-25), (*t*, C-23, C-30), 125.8 (*d*, C-12), 22.4 (*q*, C-29),79.1 (*d*, C-3), 52.4 (*s*, C-5), 23.5 (*t*, C-16), 55.2 (*d*, C-18), 24.0, 23.9 (*t*, C-11), 27.4 (*t*, C-2), 17.2 (*q*, C-26), 18.3 (*t*, C-6), 24.5 (*q*, C-27), 29.4 (*t*, C-15), 47.9 (*s*, C-17), 30.5 (*d*, C-19), 47.4 (*d*, C-9), 39.6 (*s*, C-8), 42.0 (*s*, C-14), 37.0 (*t*, C-22), 38.5 (*t*, C-1), 37.1 (*s*, C-10), 33.2 (*t*, C-7), 30.3 (*d*, C-20), 27.5 (*t*, C-21), 176.2 (*s*, C-28).

3.1.3. Spectral Data of Compound ***(3)****.* EI-MS: *m/z*: 414 $[M]^+$, 371, 138, 329, 273, 315 and 222. HR-EI-MS: *m/z*: 414.3857 ($C_{29}H_{50}O$, 414.3861). ^{1}H-NMR: ($CDCl_3$, 400 MHz) (δ ppm) 0.66 (3H, *s*, H-18), 0.78 (3H, *d*, $J = 6.0$ Hz, H-27), 0.81 (3H, *d*, $J = 6.2$ Hz, H-26), 0.98 (3H, *s*, H-19), 3.35 (1H, *m*, H-3), 0.82 (3H, *t*, $J = 7.5$ Hz, H-29), 0.90 (3H, *d*, $J = 6.5$ Hz, H-21), 5.32 (1H, br. *s*, H-6). ^{13}C-NMR: ($CDCl_3$, 125 MHz) (δ ppm) 11.8 (C-18), 121.3 (C-6), 142.0 (C-5), 26.2 (C-16), 70.3 (C-3), 56.9 (C-14), 56.7 (C-17), 34.0 (C-22), 50.3 (C-9), 32.1 (C-8), 49.0 (C-24), 42.3 (C-13), 39.9 (C-12), 40.2 (C-4), 37.3 (C-1), 37.1 (C-10), 31.6 (C-7), 28.9 (C-23, C-25), 28.6 (C-2), 22.8 (C-28), 25.3 (C-15), 20.1 (C-26), 21.1 (C-11), 19.1 (C-19), 19.3 (C-27), 18.6 (C-210), 12.0 (C-29).

3.1.4. Spectral Data of Compound ***(4)****.* HR-EI-MS *m/z*: 412.3920. Calcd. for $C_{29}H_{48}O$, 412.3926 EI-MS (rel. int. %) *m/z*: M^+ 412 (7), 270 (22), 379 (28), 394 (20), 397 (12), 273 (30), 327 (60), 300 (67), 369 (35), 351 (70), 301 (18). ^{1}H-NMR ($CDCl_3$, 400 MHz) (δ ppm) 0.65 (3H, *s*, Me-18), 0.80 (3H, *s*, Me-19), 0.81 (3H, *d*, $J = 6.5$ Hz, Me-27), 0.84 (3H, *t*, $J = 7.0$ Hz, Me-29), 0.90 (3H, *d*, $J = 6.5$ Hz, Me-21), 5.33 (1H, *m*, H-6), 5.02 (1H, *m*, H-3), 5.15 (1H, dd, $J = 15.1$, 8.4 Hz, H-22), 0.83 (1H, *d*, $J = 6.6$ Hz, Me-26), ^{13}C-NMR ($CDCl_3$, 100 MHz) (δ ppm) 12.0 (C-29), 129.4 (C-23), 19.4 (C-19), 21.0 (C-11), 31.8(C-2), 32.0 (C-25), 42.4(C-13), C-24), 39.7, 25.4 (C-28), 24.4 (C-15), 121.7 (C-6), 51.3 ((C-12), 71.9 (C-3), 28.9 (C-16), 40.5 (C-20), 50.3 (C-9), 42.2 (C-4), 32.2 (C-8), 21.2 (C-27), 57.0 (C-14), 56.0 (C-17), 36.6 (C-10), 37.4 (C-1), 31.9 (C-7), 21.1 (C-21), 19.0 (C-26), 138.4 (C-22), 12.4 (C-18), and 140.9 (C-5).

3.2. Antimicrobial Activities

3.2.1. Antibacterial Potential. The result of the antibacterial activity shows that methanol extract of *Nepeta distans* shows the maximum region of inhibition (33 mm) against *Klebsiella pneumoniae* at 300 mg/ml, while the lowest inhibition of 22.5 mm was shown against *Escherichia coli* at 100 mg/ml (Table 1). Similarly, the chloroform extract of *Nepeta distans* showed the highest zone of inhibition (30 mm) against *Staphylococcus aureus* 300 mg/ml, while the lowest zone of inhibition (21 mm) was shown against *E. coli* at 100 mg/ml (Table 1). The methanol extract showed the lowest MIC values (2 μg/ml) against *E. coli* and *pathovar,* and the chloroform extract showed the lowest MIC values (2 μg/ml) against *S. aureus* (Table 2).The MIC values are shown in Table 2.

3.2.2. Antifungal Activity. The result of the antifungal activity show that methanol extract of *Nepeta distans* show the highest zone of inhibition (35 mm) at 300 mg/ml, while the lowest zone of inhibition (22.5 mm) was shown by *Fusarium solani* at 100 mg/ml (Table 3). Similarly, the chloroform extract of the *Nepeta distans* showed the highest zone of inhibition (31 mm) against *Penicillium chrysogenum* at 300 mg/ml, while the lowest zone of inhibition (23 mm) was

Table 1: Antibacterial activity of the methanol extract and chloroform fraction of *N. distans*.

Test organism	Positive control	Negative control	Methanol extract (mg/ml)			Chloroform fraction (mg/ml)		
Concentration Zone of inhibition (mm)			100	200	300	100	200	300
E. coli	10	0	22.5	26.5	27	21	25	28
Pathovar	10	0	24	30	29	24	29	27
S. aureus	10	0	24.5	29	32	22	27	30
K. pneumoniae	10	0	25.5	29	33	23	29	28

Table 2: MIC values (gm/ml) of the antibacterial activity of methanol extract and chloroform fraction of *N. distans*.

Test organism	Methanol extract (mg/ml)	Chloroform fraction (mg/ml)
E. coli	2	4
S. aureus	6	2
K. pneumoniae	4	6
Pathovar	2	4

Table 3: Antifungal activity of the methanol extract and chloroform fraction of *N. distans*.

Test organism	Positive control	Negative control	Methanol extract (mg/ml)			Chloroform extract (mg/ml)		
Concentration Zone of inhibition (mm)			100	200	300	100	200	300
A. flavus	10	0	27.5	29	26	28	25	21
A. fumigatus	10	0	25.5	24.5	23	27	29	24
F. solani	10	0	27	24.5	22.5	30	27	22
A. Niger	10	0	33	28.5	24.5	28	29	23
P. chrysogenum	10	0	35	32	28.5	31	30	28
A. tamarii	10	0	32	29	25	30	27	22

shown by *Fusarium solani* at 100 mg/ml (Table 3). The methanol extract showed the lowest MIC values (2 μg/ml) against *Aspergillus niger*, and the chloroform extract showed the lowest MIC values (2 μg/ml) against *Aspergillus tamarii*. Both the methanol extract and the chloroform fraction showed moderate activities against *Aspergillus flavus* and *Aspergillus fumigatus* at all three concentrations (Table 4). The MIC values are displayed in Table 4.

3.3. Phytochemical Screening. Phytochemical screening showed that both methanol extract and chloroform fraction showed the presence of alkaloids, flavonoids, saponins, glycosides, proteins, and oil (Table 5).

Screening the crude extract for phytochemical analysis is an imperative means for finding the secondary metabolites that exist in the medicinal plant because these phytochemicals are counted responsible for therapeutic application. These phytochemicals responsible for that it has been reported that saponins are among the most important compounds responsible for antidiabetic, antispasmodic, antitumor, anthelmintic, antimicrobial, phytotoxic, cytotoxic, and antioxidant potential [38].

Nepeta distans is a rich source for natural products; many constituents isolated and reported in literature are β-sitosterol, eugenol, ursolic acid, thymoquinone, oleanolic acid, nepedinol, netidiol, markhamioside F, and nepatanol [39].

Table 4: MIC values (gm/ml) of the antifungal activity of the methanol extract and chloroform fraction of *N. distans*.

Test organism	Methanol extract (mg/ml)	Chloroform extract (mg/ml)
A. flavus	2	4
A. fumigatus	6	2
F. solani	4	6
A. Niger	2	4
P. chrysogenum	4	2
A. tamarii	6	4

Table 5: Qualitative phytochemical analysis of *Nepeta distans*.

Class of phytochemical	Methanol extract	Chloroform fraction
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Proteins	+	+
Oils	+	+
Glycosides	+	+

A study confirmed that the genus *Nepeta* species is an important source of nutrients and showed immunomodulatory potential. *Nepeta* is multiregional genus and a variety of compounds are reported from it in literature, such as β-amyrin, glutinol, stigmasterol glucoside, 9β, 10α-cleroda-3, 13 (16),14-trien-18-oic, 5, 4′-dihydroxy-3, 6, 7-

trimethoxyflavone, (−)-6β-hydroxy-15, 16-epoxy-5β, 8β ,and stigmasterol [40–42]. The isolation and characterization of the compounds 1–4 from *Nepeta distans* are in complete agreement with the findings of [40–42]. Similarly, many species of the genus *Nepeta* are used traditionally in folklore medicine for the treatment of liver and kidney problems, to treat dysentery and teeth problems, and are used as sedative agents and stimulants, and they are also used as febrifuge, antiasthmatic, antispasmodic, diaphoretic, and diuretic.

Essential oil and crude extracts of *Nepeta* genus have showed antimicrobial potential, significant antiglycation activity, anti-inflammatory potential, and serum lipids reduction potential [43]. Our findings of antimicrobial potential of *Nepeta dastans* are again completely in agreement with the published literature [43]. Another published research showed that the *Nepeta* species possessed multibiological potentials such as antidiabetic, acetylcholinesterase inhibitory, antiatherosclerotic, analgesic, anti-ociceptive, antimalarial, antileishmanial, antioxidant, antimicrobial, anthelmintic, antiglycation, antiplatelet aggregation, cardioprotective effect, cytotoxic, anticancer and apoptotic, genotoxic, immunomodulatory, hepatoprotective, insecticidal and insect repellent, trypanocidal, phytotoxic, nematocidal, dyslipidemia, and larvicidal [44].

4. Conclusion

The present study shows that *Nepeta distans* possess bioactive phytochemicals such as flavonoids, alkaloids, and tannins , and its methanol extract and chloroform fraction showed antimicrobial potential. The published literature showed multiple biological activities of *Nepeta species*. Therefore, it is highly recommended to explore *Nepeta distans* for more phytochemical as well as biological investigation.

Data Availability

The data used to support this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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