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Research Article

Biological Potential of *Nepeta distans* Belonging to Family Labiatae

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ABSTRACT
The aim of this study was to assayed the biologically active fractions of *Nepeta distans* against platelet aggregation, antiglycation, cytotoxicity (brine shrimp lethality), Phytotoxicity, antibacterial and antifungal. Both the fractions derived from the aerial parts of *Nepeta distans* were screened for various in vitro biological activities. Chloroform as well as hexane fractions inhibited AA-induced and PAF-induced platelet aggregation in a dose dependent fashion. Both the fractions were also found to possess excellent phytotoxicity against *Lemna minor L.* These fractions did not display any significant brine shrimp lethality, insecticidal, antibacterial and antiglycation activities. However, chloroform fraction showed low antibacterial activity against *S. typhi*.
Keywords: Labiatae, *Nepeta distans* Antiplatelets aggregation, antiglycation, cytotoxicity (brine shrimp lethality), phytotoxicity, and antimicrobial activity.

INTRODUCTION

The genus *Nepeta* (Lamiaceae) is a large family comprises about 400 species, most of which grow wild in Central and Southern Europe, the North Africa and Central and Southern Asia. A lot of species of this genus are used in folk medicine for the antiseptic and astringent properties as topical remedy in children cutaneous eruptions, snakes and scorpion bites; orally, they are utilized as anti-tussive, anti-spasmodic, anti-asthmatic, febrifuge and diuretic [1,2,3,4,5,6,7]

Moreover, anti-bacterial, fungicidal and anti-viral activities have been attributed to nepetalactones, iridoids contained in several *Nepeta* species [6,7]. Some endemic species of the Southern Greece are utilized in traditional medicine. In particular, *Nepeta parnassica* Heldr. and Sart. ex Boiss. and *Nepeta troodi* Holmboe fresh leaves are chewed to alleviate toothache and a leaves alcoholic macerate is efficacious for treatment of contusions and rheumatic pains [8].

In many countries, several *Nepeta* species are

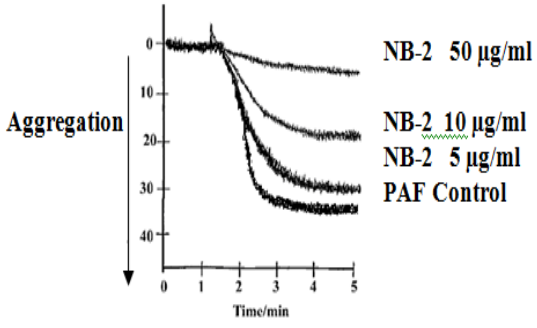

Figure: 1a. Tracings of inhibition of the platelet aggregation by **(NB-2)** against AA. Control is aggregation curve obtained by adding AA (1.8mM) and was taken as 100% aggregation. (IC_{50} value = AA =13 µg/ml)

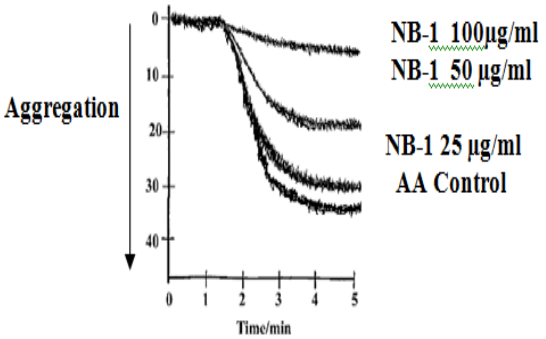

Figure: 1b. Tracings of inhibition of the platelet aggregation by **(NB-1)** against AA. Control is aggregation curve obtained by adding AA (1.8mM) and was taken as 100% aggregation. (IC_{50} value = AA= 53 µg/ml)

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Table1a: Phytotoxicity of hexane fraction (**NB-1**)

Name of Plant	Conc.of Compound (µg/mL)	No. of Fronds		% Growth Regulation	Conc.of Std. Drug(µg/mL)
		Sample	Control		
Lemna	1000	0		100	
Minor	100	0		100	0.015
	10	8		39.9	

Remarks: Hexane fraction **(NB-1)** shows significant activity at the two highest doses.

Table 1b: Phytotoxicity of chloroform fraction (**NB-2**)

Name of Plant	Conc.of Compound (µg/mL)	No. of Fronds		% Growth Regulation	Conc.of Std. Drug(µg/mL)
		Sample	Control		
Lemna	1000	0		100	
Minor	100	3		84.5	0.015
	10	5		62.5	

Remarks: Chloroform fraction **(NB-2)** shows significant activity at the two highest doses.

used in the traditional medicines. They are used as

laxative, to treat dysentery, kidney, liver diseases and

teeth troubles [9]. It is also used as diuretic, diaphoretic, vulnerary, antispasmodic, antiasthmatic, tonic, febrifuge and sedative agents [9, 10, 11, 12]. The genus is also reported to possess biological activities especially reduction of serum lipids and anti-inflammatory effects [13]. Biological activities of *Nepeta juncea* such as platelet aggregation, antiglycation, cytotoxic, phytotoxic and antimicrobial are also reported by Hussain *et al*., (2009) [14].

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

Therefore, platelet aggregation, antiglycation, cytotoxicity (brine shrimp bioassay), phytotoxicity (*Lemna* bioassay), antibacterial, insecticidal and antifungal activities were performed for methanol extract and subsequent fractions of the whole plant of *Nepeta distans*.

MATERIALS AND METHODS

Plant Material

The whole plant of *Nepeta distans* was collected from Parachinar Kurram Agency, KPK(NWFP), Pakistan, in June 2005, and was identified by Prof. Dr. Jehandar Shah, Islamia College Peshawar. Herbarium specimens were deposited in the herbaria of Department of Botany, Kohat University of Science and Technology (KUST), Kohat, KPK(NWFP, Pakistan.

Extraction

The whole plant of *N. distans* was dried in dark, chopped and ground to coarse powder. The powdered plant (8.5 kg) was initially extracted with methanol (7 days x 3) at room temp. The combined methanolic extract was evaporated under reduced pressure leaving behind a greenish, syrup residue (195 g). The methanol extract was partitioned in various fractions through separating funnel. It was partitioned into Chloroform (55 g), ethyl acetate (25 g) and aqueous fractions (15) successively.

METHODOLOGY

Platelet Aggregation Activity

Blood was taken from health human volunteers who have not taken any medication during the last 2 weeks. Blood samples was mixed with 3.8 % (w/v) sodium citrate solution (9:1) and centrifuged at 260 g for 15 min at 20 °C to obtain platelet rich plasma (PRP). The remaining blood sample was centrifuged at 1200 g for 10 min to obtain platelet poor plasma (PPP). Platelet count was determined by phase contrast microscopy and all aggregation studies were carried out at 37 °C with PRP having platelet counts between 2.5 and 3.0 x 10^8 mL^{-1} of plasma [15, 16].

Aggregation was monitored by dual-channel Lumi aggregometer (Model 400 Chronolog Corporation, Chicago, USA) using 0.45 ml aliquots of PRP (Ross, R, 1999). The final volume was made up to 0.5 ml with the aggregating agent, dissolved either in normal saline or appropriate vehicle known to be devoid of any effect on aggregation. Aggregation was induced with AA (1.8 mM) or PAF (0.8 µM). The anti-aggregatory effect of compound (extract) was studied by addition of aggregation agents (AA and

Table 2a: In vitro antifungal activity of hexane fractions of **(NB-1)** compared to the reference standard drug, Miconazole.

Plant Extract 100mg/ml	Diameter of zones of inhibition in mm					
	Trichphyton longifusus	***Aspergillus flavus***	***Candida albicans***	***Microsporum canis***	***Fusarium solani***	***Candida glabrata***
Hexane	-	-	-	-	-	-
Std. drug	70	20	110.8	98.4	73.25	110.8

Table 2b: In vitro antifungal activity of hexane fractions of **(NB-2)** compared to the reference standard drug, Miconazole.

Plant Extract 100mg/ml	Diameter of zones of inhibition in mm					
	Trichphyton longifusus	***Aspergillus flavus***	***Candida albicans***	***Microsporum canis***	***Fusarium solani***	***Candida glabrata***
Chloroform	-	-	-	20	-	-
Std. drug	70	20	110.8	98.4	73.25	110.8

Table 3: Antibacterial activity of chloroform fraction (**NB-2**)

Name of Bacteria	Zone of Inhibition of sample (mm)	Zone of Inhibition of Std. Drug (mm)
Eschericha coli	-	30
Bacillus subtilis	-	37
Shigella flexenari	-	36
Staphylococcus aureu	-	26
Pseudomonas aeruginosa	9	32
Salmonella typhi	2	30

Remarks: Chloroform fraction **(NB-2)** shows low activity against S. typhi and non significant antibacterial activity against Ps. Aeruginosa

PAF). The resulting aggregation was recorded for 5 min after challenge, by the change in light transmission as a function of time.

Antimicrobial Bioassay

The antibacterial activity was determined by agar well diffusion method. A loopful of a 104-106 suspension of 24 h old broth of each bacterium was streaked on the surface of Mueller- Hinton agar (BBl-USA) plates. Wells were dug in the agar with the help of sterile dimethyl sulfoxide (DMSO). Dilutions of the stock solution containing 50,100, 150 and 200 µg were prepared in DMSO and 100 µl of each dilution was added in the respective wells. The plates were

then incubated at 37 °C for 24 h and zone of inhibitions were measured in millimeters (mm) and compared with the control [17]. Antibacterial activity was studied against *Eschericha coli, Bicillus subtilis, Shigella flexenari, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi*. Ampicillin, Tobramycin and Amoxacilline were used as standard drugs. The samples of *Nepeta distans* were subjected to antibacterial screening.

The antifungal activity of the crude extracts (chloroform and hexane) of *Nepeta distans* was determined by measuring the minimum inhibitory concentration of the extract using agar well diffusion assay. The assay was carried out according to the method of Hufford *et al* 1975 [18]. The extract was tested at final concentration of 78- 50 %. A 24 h culture of *Candida albicans*, diluted to give a final concentration of 10^5 cells per ml, was used, while standards spore suspensions of 10^3 ml from the other test fungi were utilized in the Minimum Inhibition Concentration (MIC) assays. After inoculating the Sabouraud's dextrose agar (previously incorporated with the desired extract concentration with fungi, the plates were incubated at 30 °C for 5 days. Control plates containing each of the test fungi separately without the addition of the anti-microbial extracts were similarly set up. The minimum inhibitory concentration was regarded as the lowest concentration of the extract that did not permit the growth of any of the test fungi after the period of incubation.

Phytotoxicity Screening

This test was performed according to the modified protocol of McLaughlin *et al.* (1988) [19]. The test fractions were incorporated with sterilized E-medium at different concentrations i.e. 10, 100, 1000 µg/ml in methanol. Sterilized conical flasks were inoculated with fractions of desired concentrations prepared from the stock solution and allowed to evaporate overnight. Each flask was inoculated with 20 ml of sterilized E-medium and then ten *Lemna minor* each containing a rosette of three fronds were placed on media. Other flasks were supplemented with methanol serving as negative control and reference inhibitor i.e. Parquet serving as positive control. Treatment was replicated three times and the flasks incubated at 30°C in Fisons Fi-Totron 600H growth cabinet for seven days, 9000 lux intensity, 56+10 rh (relative humidity) and 12 hours day length. Growth of *Lemna minor* in fraction containing flask was determined by counting the number of fronds per dose and growth inhibition was calculated with reference to negative control [19].

Table 4: Antiglycation activity of chloroform and hexane fractions (**NB1**)

Sample Code No	Conc. (mM)	% Inhibition	IC_{50}^{+} SEM [mM]
Hexane Fraction (**NB1**)	1 mg/100µl	24.3	
Chloroform Fraction (**NB2**)	1 mg/100µl	6.6	

Remarks: Both the fractions are less significant against Glycation.

Antiglycation Activity

BSA (Bovine Serum Albumin) as 10 mg/ml, dissolved in 67 mM phosphate buffer (pH: 7.4).

Table 5a: Brine shrimp lethality of hexane fraction (**NB1**)

Dose (µg/mL)	No. of Shrimps	No. of Survivors
1000	30	18
100	30	24
10	30	26
LD_{50}(µg/ml)		-

Remarks: No brine shrimp lethality of hexane fraction (NB1)

Table 5b: Brine shrimp lethality of chloroform fraction (**NB2**)

Dose (µg/mL)	No. of Shrimps	No. of Survivors
1000	30	25
100	30	27
10	30	27
LD_{50}(µg/ml)		-

Remarks: No brine shrimp lethality of hexane fraction (NB1)

Glucose as 50 mg/ml, dissolved in 67 mM phosphate buffer (pH: 7.4) of Buffer (pH: 7.4). 3 mM Sodium azide in required quantity of Buffer to inhibit bacterial growth. Using 3 mM concentrations of new compounds along with standard inhibitors. Incubating the prepared sample (60 □l in each well) in the incubator for a week at 370 °C. After incubation 96-well plate for a week, samples were taken out and cooled at room temperature. Then 6 µl of 100% TCA (Trichloro acetic acid) was added to each well, supernatant containing unbounded glucose, inhibitor and interfering Substances were removed after agitation and centrifugation at 14000 rpm for 4 minutes, pellets were obtained at the bottom of the wells. Then solvent was removed from each well, and 60 □l of PBS (pH: 10) was added to dissolve the pellets. The comparison of fluorescence intensity at 370 nm Excitation and Emission at 440 nm was

obtained by using spectrofluorimeter. Rutin was used as the standard inhibitor.

Brine Shrimp Lethality Bioassay

The procedure of Mayer et al was adopted to determine the lethality of plant extracts to brine

shrimp. Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds [20]. Using the protocol of Meyer *et al* [20], brine shrimp (*Artemia salina* larvae) eggs were hatched in a

shallow rectangular plastic dish, filled with artificial seawater, which was prepared by mixing a commercial salt mixture (Instant Ocean, Aquarium System, Inc., Mentor, OH, USA) with double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. An approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the smaller compartment was opened to ordinary light. After two days a pipette collected naupil from the lighted side. A sample of the test fraction was prepared by dissolving 20 mg of each fraction in 2 ml of methanol. From this stock solution, 1000,100 and 10 µg/ml was transferred to 12 vials; three for each dilution, and three vials were kept as control having 2 ml of methanol only. The solvent was allowed to evaporate overnight. After two, when shrimp larvae were ready, I ml of sea water was added to each vial along wit 10 shrimps and the volume was adjusted with sea water to 5 ml per vial. After 24 hours, the number of surviving shrimps counted. Data was analyzed by a Finney computer program to determine the LD_{50} [21].

Insecticidal Activity

Concentration of test sample (each fraction) (1571.33 µg/cm^2) was prepared. Permethrin (coopex^{TM}) was used as standard drug with 235.71 µg/cm^2 concentration. The stored grain pests are reared in the laboratory under controlled temperature and humidity, so that the insects of uniform age and size were available for the experiments. Ten pairs of insects are reared in 9.0 cm diameter and 11.0 cm high plastic bottles containing 250 g of breeding media. Then bottles are covered with muslin cloth tied by means of rubber bands, or small jars or wide mouthed bottles sealed with filter paper (Whatman

No. 29, black) and paraffin wax (to prevent contamination) are suitable. The media should be sterilized at 60 ºC for one hour.

The insects are exposed to test sample (each fraction) by contact method using filter paper. 1 ml of different concentration of every fraction is applied by micropipette to 90 mm diameter filter papers and then placed in the petri dishes. After that adult insects of same size and age in each batch are transferred to Petri dishes. A check batch is treated with solvent for determination of solvent effect. A control batch is kept for determination of environmental effects. Another batch supplemented with reference insecticides e.g. coopex and deeis (synthetic Pyrethroids) are used. All these are kept without food throughout 24 hours exposure period. Mortality counts are done after 24 hours exposure period. lC_{50} values then determined by probit mortality curve that is drawn on log-log graph paper.

Table 6a: Insecticidal activity of hexane fraction (**NB-1**)

Name of Insects	% Mortality		Sample
Tribolium castaneum	100	-	0
Sitophilus oryzae	-	-	-
Rhyzopertha dominica	100	-	0
Callosbruchus analis	100	-	40
Trogoderma granarium	-	-	-

Remarks: Hexane fraction **(NB-1)** shows moderate activity against *C.analis*.

Table 6b: Insecticidal activity of chloroform fraction (**NB-2**)

Name of Insects	% Mortality		Sample
Tribolium castaneum	100	-	0
Sitophilus oryzae	-	-	-
Rhyzopertha dominica	100	-	0
Callosbruchus analis	100	-	20
Trogoderma granarium	-	-	-

Remarks: Chloroform fraction **(NB-2)** shows non-significant activity against *C. analis.*

RESULTS AND DISCUSSION

Antiplatelets aggregation

The aggregation activity shown by chloroform fraction (NB2) was confirmed in the dose-dependent studies, which inhibited AA induced platelet aggregation in a dose dependant fashion (Fig 1a). In this study, concentration of chloroform (NB2) fraction required to inhibit AA induced human platelet aggregation by 50% (IC_{50}) was found to be 13 µg/mL. This activity of the chloroform fraction (NB2) seems to be independent of its COX inhibitory activity. According to Hussain *et al* (2009) [14], chloroform (NJC) fraction of *N. juncea* did not show any effects on PAF induced platelet aggregation but water fraction (NJW) and n-hexane (NJH) were effective against AA and (IC_{50}) AA= 13 µg/ml, (IC_{50} value= AA=48 µg/ml) respectively (%).

The hexane fraction (NB1) was also effective in inhibiting PAF-induced platelet aggregation in a dose related manner with IC_{50} of 53 µg/ml (Fig 1b). Since AA produces its aggregating effect through cyclooxygenase (COX) enzyme, it is likely that hexane (NB1) fraction mediates its anti-aggregatory effect via inhibition of platelet COX.

This indicates the presence of anti-aggregation activities (against AA and PAF) in the chloroform (NB2) and hexane fractions (NB1). It is not clear whether these activities are displayed by separate phytocompounds or a single compound

showing inhibition of AA and PAF-induced platelet aggregation. However (Shehnaz *et al*., 1999) [22] reported that PAF induced platelet aggregation was blocked by all the concentrations but the extract exhibited no effect on AA induced platelet aggregation. Our results showed that the inhibition of the agents, AA and PAF induced platelet aggregation was observed in chloroform and hexane fractions and therefore, platelet inhibitory constituents are concentrated in these fractions.

Phytotoxicity Screening

The herbicidal potentials of the extracts derived from the plant *Nepeta distans* were evaluated against *L. minor*. The screened samples of chloroform (NB2) and hexane (NB1) showed very good phytotoxic activity at two highest doses (Table 1a, b).

Phytotoxic studies of genus *Nepeta* are not studied in detail. However, essential oils of *N. pannonica* [60 components with 1,8-cineole (28.9%), and 4α,7ß,7α-nepetalactone (14.3%) as the major constituents] exhibited more phytotoxicity to bentgrass as compared to lettuce seeds, with 100% growth inhibition observed at 0.3, and 1.0 mg/ml, respectively [23]. Similarly n-hexane fraction (NJH) of *N. Juncae* showed significant activity at the highest dose (39.9 %) [14].

Antimicrobial Bioassays

Both hexane and chloroform fractions of *Nepeta distans* were tested against six bacteria (*Bacillus subtilis, Eschericha coli, Shigella flexneri, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Salmonella typhi*) and six fungi (*Aspergillus flavus, Candida albicans, Candida glabrata, Fusarium solani, Microsporum canis,* and *Trichphyton longifusus*). No significant inhibitory effect of these fractions could be observed against these bacteria and fungi (Table 2a, b). With exception, chloroform fraction showed weak antibacterial activity against *S. typhi* (27.03 %) (Table 3).

Similar results were reported by Hussain *et al*., 2009 [14] when all the fractions of *N. juncea were tested* against six bacteria (*B. subtilis, E. coli, S. flexneri, S. aureus, P. aeruginosa,*and *S. typhi*) and six fungi (*Aspergillus flavus, Candida albicans, Candida glabrata, Fusarium solani,Microsporum canis,* and *Trichphyton longifusus*). No significant inhibitory effects of these fractions were observed against these bacteria and fungi. However, the n-butanol fraction showed weak antibacterial activity against *B. subtilis* (27.0%), *S. flexneri* (30.7%) and *S. aureus* (42.3%). The chloroform fraction of *N. juncae* showed 25% antibacterial activity against *S. typhi* only.

Khan *et al*. (2005) [24] had used the same bacterial and fungal strains as test against crude extracts of *P. emodi* but they also found no significant antibacterial or antifungal activities against these strains. However, essential oils of *N. juncea*, with major constituent of nepetalactone, have shown antifungal and antibacterial properties [25, 26, 27, 28]. Similarly essential oils (major constituent nepetalactone) of *N. cadmia* [29] and *Nepeta rtanjensis* were active against five bacterial strains but not against a fungal strain, while *Aspergillus niger*[30] *Nepeta crispa* [31] *Nepeta sibthorpii* [32] *N. cataria* [33] and *Nepeta ispahanica* [34] showed antimicrobial and antifungal activities.

Antiglycation Activity

Both fractions of chloroform and hexane were tested against the formation of Advanced Glycation Endproducts (AGEs), but the results were not significant. However hexane showed 24.3 % inhibition at concentration of 1 mg/100µl, where as chloroform inhibition was 6.3 % at the same concentration respectively (Table 4).

Brine Shrimp Lethality Bioassay

Brine shrimp lethality is the simple bioassay useful for screening large number of extracts in the drug discovery process from the Indian Medicinal plants. Both the fractions of chloroform (NB2) and hexane (NB1 obtained were screened for brine-shrimp lethality bioassay by using the protocol of Meyer *et al*., 1982 [20]. All the values were calculated by using Finny computer program [21]. No toxic effects were found in these fractions.

According to Hussain *et al* (2009) [14] n-hexane fraction of *N. Juncae* did not show any significant cytotoxic activity. Khan *et al*. (2005) [24] had studied brine shrimp lethality for ethanolic extracts of *Paeonia emodi* that did not show significant toxic effects at 1000, 100 and 10 ug/ml while the IC_{50} value was also higher than 1000 µg/ml.

Both fractions were less significant against the screened organisms. However hexane fraction of *Nepeta distans* with LD_{50} value of 18 µg/ml where as chloroform fraction with LD_{50} showed 25 µg/ml (Table 5a, b). The extracts with LC_{50} values higher than 200 mg/l in the brine shrimp test can be considered inactive [35].

Insecticidal Activity

Chloroform and hexane fractions of *Nepeta distans* were evaluated against five insects (*Tribolium castaneum, Sitophilus oryzae, Rhyzopertha dominica, Callosbruchus analis and Trogoderma granarium*). No significant inhibitory effect of chloroform fraction was observed against these insects. However hexane fraction showed moderate activity against *Callosbruchus analis* with LC_{50} of 40 (Table 6a, b).

CONCLUSION

The results suggest that both fractions have variable effects in different bioassays. The chloroform as well as hexane fraction showed highest inhibition in platelet aggregation which may be the potential fraction for characterization and isolation of targeted compounds. Phytotoxic studies of hexane fraction against *Lemna minor* showed 100 % at the highest dose, while chloroform fraction showed low antibacterial activity against *S. typhi.* In case of antimicrobial activities, the fractions did not exhibit any inhibitory effect against bacterial and fungal strains. Thus essential oils may contain the targeted compound for antimicrobial activity.

REFERENCES

1. Lewis, W.H., 1977. Medical Botany (Plants Affecting Man's Health). Wiley-Interscience Publication/John Wiley & Sons, New York, p. 257.
2. Perry, L.M., 1980. Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses. MIT Press, Cambridge, MA, p. 620.
3. Moerman, D.E., 1982. Geraniums for the Iroquois: A Field Guide to American Indian Medicinal Plants. Reference Publications, Inc., Algonac, Michigan, pp. 43–45.
4. Duke, J.A., Ayensu, E.S., 1985. Medicinal Plants of China, vol. 2. Reference Publications, Inc., Algonac, Michigan, p. 376.
5. Bezanger-Beauquesnes, L., Pinkas, M., Torck, M., Trotin, F., 1990. Plantes M´edicinales des R´egions Temp´er´ees. Maloine, Paris, pp. 294–295.
6. Bourrel, C., Perineau, F., Michel, G., Bessiere, J.M., 1993. Catnip (*Nepeta cataria* L.) essential oil: analysis of chemical constituents, bacteriostatic and fungistatic properties. Journal of Essential Oil Research 5, 159.
7. Sattar, A., Bankova, V., Kujumgiev, A., Galabov, A., Ignatova, A., Todorova, C., Popv, S., 1995. Chemical composition and biological activity of leaf exudates from some Lamiaceae plants. Pharmazie 50, 62–65.
8. Arnold, N., Bellomaria, B., Valentini, G., Yanniou, C., Arnold, H.J., 1993a. ´ Etude anatomique et phytochimique de *Nepeta troodi* Holmboe (Labi´ees) end´emique de Chypre. Plantes M´edicinales et Phytoth´erapie XXVI, 52–63.
9. Baser KHC, Kirimer N, Kurkcuoglu M, Demirci B. 2000. Essential oils of *Nepeta* species growing in Turkey. *Chem Nat Compd* **36:** 356–359.
10. Dabiri, M. and Sefidkon, F. 2003. Composition of essential oil of *Nepeta crassifolia* Boiss Buhse. *Flav Fragr J* **18:** 225–227.
11. Rapisarda A, Galati EM, Tzakou O, Flores M, Miceli N. 2001. *Nepeta sibthorpii* Bentham (Lamiaceae): Micromorphological analysis of leaves and flowers. *Farmaco* **56:** 413–415.
12. Zargari A. 1990. *Medical Plants*, 4, Tehran University Publications, Tehran. Pp.106–111. Agarwal OP, Khanna DS, Arora RB. 1978. Studies of anti-atherosclerotic action of *Nepeta hindostana* in pigs. *Arterry* **4:** 487–496.
13. Prokopenko, S. A. and Spiridonov AV. 1985. Betulin from Transcaucasian clover (*Nepeta*). *Farm Zhurnal* **6:** 70.
14. Javid H, Nargis J, Gilani S. A, Ghulam A and Sagheer A, 2009. *Afr. J of Biotechnology*, **8** (6), pp. 935-940.
15. Saeed, S. A. Connor J. D. Imran, Q. J, Tasneem, S. Ahmed, S. Mesaik, M.A, Choudhary, M. I. 2007. *Pharmacol Rep. 59,* **2**, 238-243.
16. Shah, B. H. Shamim, G. Khan, S. Saeed, S. A. 1996. *Biochem. Mol. Biol. Int.* **38**, 1135-41.
17. Att-ur-Rehman, 1991. *Studies in Natural*

Product Chemistry, 383 (Part B) Elsevier Science Publishers, B.V,Netherlands,

18. Hufford CD, Funderburk JM, Morgan JM, Robertson LW. 1975. Two antimicrobial alkaloids from heartwood of *Liriodendron tulipifera*. *I.J.pharm. Sci*., **64**, 789-792
19. McLaughlin JL,. 1988. Proceedings of NIH Workshop, Bioassay For Discovery of Antitumoral, Antiviral Agents from Natural Sources, Bethesda, p.22.
20. Mayer, B. N. Ferrigni, N. R. Putnam, J. E. Maclaughlin, 1982. *J. L Planta Medica,* **45**, 31,
21. Finney, D. J. 1971. "Probit Analysis", Cambridge University Press, Cambridge , 3rd ed., 333.
22. Shehnaz D, Hamid F, Baqai FT, Ahmad VU. 1999. Effect of the crude extract of *Cestrum parqui* on Carrageenin-induced rat paw oedema and aggregation of human blood platelets. *Phytother Res* **13:** 445–447
23. Kobaisy M, Tellez MR, Dayan FE, Mamonov LK, Mukanova GS, Sitpaeva GT, Gemejieva NG. J Essential Oil Res 2005; 17: 704–707.
24. Khan T, Ahmad M, Khan H, Khan MA. 2005. Biological activities of aerial parts of *Paeonia emodi* Wall. *African Journal of Biotechnology* **4:** 1313–1316.
25. Inoue S, Uchida K, Yamaguchi H, Miyara T, Gomi S, Amano M. 2001. Karakoram-Himalaya district and their antifungal activity by vapor contact. *J. Essential Oil Research* **13**: 68–72.
26. Tripathi P, Dubey NK, Shukla AK. 2008. Use of some essential oils as post-harvest botanical fungicides in the management of grey mould of grapes caused by *Botrytis cinerea*. *World J Microbiology & Biotechnology* **24**: 39–46.
27. Tripathi P, Dubey NK, Banerji R, Chansouria JP. N. 2004. Evaluation of some essential oils as botanical fungitoxicants in management of post-harvest rotting of citrus fruits. *World J Microbiology & Biotechnology* **20**: 317–321.
28. Kobaisy M, Tellez MR, Schrader KK, Wedge DE, Sitpaeva GT, Gemejieva NG, Mukanova GS, Mamonov LK. 2006. Phytotoxic, antialgal, and antifungal activity of constituents from selected plants of Kazakhstan. ACS Symposium Series **927**(Natural Products for Pest Management): 142 – 151.
29. Celick A., Mercan M., Arsalan I, Davran H. 2008. Chemical composition and antimicrobial activity of essential oil from *Nepeta cadmea*. *Chemistry of Natural Compounds* **44**: 119–120.
30. Stojanovic G, Radulovic N, Lazarevic J, Miladinovic D, Dokovic D. 2005. Antimicrobial activity of *Nepeta rtanjensis* essential oil. *J Essential Oil Research* 17: 587 – 589.
31. Sonboli A, Salehi P, Yousefzadi M. 2004. Antimicrobial activity and chemical composition of the essential oil of *Nepeta crispa* Willd. From Iran. *Zeitschrift fuer Naturforschung, C: J Biosciences* **59**: 653–656.
32. Galati EM, Tzakou O, Miceli N, Pizzimenti F, Rapisarda A. 2006. Pharmacognostic screening on *Nepeta sibthorpii* Bentham. *Recent Progress in Medicinal Plants* **12**: 239–256.
33. Billerbeck VG. 2007. Essential oils and antibiotic-resistant bacteria. *Phytotherapie* **5**: 249-253.
34. Salehi P, Sonboli A, Allahyari L. 2007. Antibacterial and antioxidant properties of the essential oil and various extracts `of *Nepeta ispahanica* from Iran. *J. Essential Oil-Bearing Plants* **10**: 324–331.
35. Anderson JE, Goetz CM, McLaughlin JL, Suffness MA 1991. A blind comparison of simple bench-top bioassays and human tumour cell cytotoxicities as antitumor prescreens. Phytochem. Anal. 2: 107.