American-Eurasian J. Agric. & Environ. Sci., 7 (6): 689-692, 2010
ISSN 1818-6769

Antifungal and Immunomodulatory Potential of Nepeta Suavis

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Abstract: In the present study we are reporting the antifungal and immunomodulatory activities of various fractions of *Nepeta suavis*, such as, chloroform, hexane, butanol, ethyl acetate and aqueous. The chloroform and butanol fractions showed inhibitory action against *Trichophyton longifusus*, *Aspergillus flavus*, *Candida albicans*, *Fusarium solani and Microsporumcanis*. In case of immunomodulatory study, the chloroform fraction showed clear modulatory effect for oxidative burst of polymorphoneutrophils and ethylacetate for the whole blood, while the aqueous fraction showed moderate inhibition. Hence, both the chloroform and ethylacetate fractions of *Nepeta suavis* are recommended further for the identification and isolation of active compounds, responsible for the observed effects.

Key words: *Nepeta suavis* · Antifungal · Immunomodulatory · Labiatae

INTRODUCTION

The genus *Nepeta* is one of the largest genuses among the genera of family labiatae (Lamiaceae) having approximately 250 species distributed mainly in Southwest and Central Asia, Europe, North Africa and North America [1]. About 67 species are found in Iran and 58 in Pakistan. Members of the genus *Nepeta* are sub-shrubs, perennial or annual herbs, monoecious or dioecious and usually aromatic in nature [2]. Some of the Iranian *Nepeta* species having great interest in Iranian folk and traditional medicines and used in the treatments of various disorders such as nervous, respiratory and gastro intestinaldisease[3].

A literature survey shows that members of the genus *Nepeta* are rich with fatty acids, flavones, flavone gl ycosides, coumarins, steroids, monoterpenic lactones and eudesmane diterpenoids [4].

Recently, the use of chemicals from natural sources is gaining considerable attention for exploitation as natural fungicides. Several plant extracts have been reported to possess antifungal activities [5]. Considerable success has been achieved by plant extracts in controlling some plant diseases under field conditions [6]. The growth of several fungi is inhibited by the crude extract (water, chloroform and ethyl acetate fraction) of *Nepeta suavis* belonging to family labiatae.

There are several herbs used in the indigenous systems of medicine that may modulate the body's immune system. A variety of secondary metabolites such as flavonoids, tannins and polysaccharides have been reported to modulate the immune system [7]. In the current study, *Nepeta suavis* was evaluated for immunomodulatory activity.

The objectives of this article are to address the recent therapeutic value of *Nepeta suavis* shrub for in *vitro* antifungal activity and immunomodulatory activity which could serve as good source for the development of new antifungal as well as immunomodulatory agents and standardized phytomedicines.

MATERIALS AND METHODS

Plant Materials: Aerial parts of *Nepeta suavis* were collected from the tribal zone, Kurram Agency NWFP, Pakistan, near Afghanistan border. Herbarium specimens were deposited in the herbaria of Department of Botany, Kohat University of Science and Technology, Kohat, NWFP Pakistan and Department of Botany, Government Post Graduate Jehanzeb College, Swat Pakistan.

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The whole plant of *Nepeta suavis* was dried in dark, chopped and ground to coarse powder. The powdered plant (4 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 (120 g). The methanol extract was partitioned in various fractions through separating funnel. It was partitioned into hexane (27g) chloroform (30g), ethyl acetate (20g), butanol (25g) and water (18g) fractions successively.

Experimental

Antifungal Activity: For antifungal study each extract was re-suspended in DMSO at a concentration of 100 mg/ml and stored in a refrigerator till further used. *Antifungal activities of* the extracts were evaluated by means of agar well diffusion assay. The assay was carried out according to the method of Hufford *et al.* [8]. Sabouraud dextrose agar (Difco) was used for the growth of fungus. Media with acidic pH (pH 5.5 to 5.6) containing relatively high concentration of glucose (40%) is prepared by mixing (SDA) Sabouraud dextrose agar and distilled water and autoclaved at 121 °C for 15 minutes. Twenty ml of molten (45°C) SDA medium was aseptically transferred into each 100mm×15mm sterile Petri dish. All these dishes were inoculated with 4mm diameter piece of inoculums removed from a seven days old culture of fungus [9]. For counting of colonies another 4mm culture (fungi) were suspended in normal saline to make volume up to 1ml and then counted with help of heamocytometer (neubar chamber). Once the agar was hardened, 11mm wells were bored using a sterile cork borer. Then 0.1ml (100 μl) from each stock solution of the extracts having final concentration of 100mg/ml was placed in each the well and the plates were incubated at 27-29°C for 7-10 days. Two wells in each Petri dish were supplemented with DMSO and reference antifungal drug, amphotericin-B (0.2mg/ml) dissolved in DMSO (sigma) serve as negative and positive control respectively. The tests were carried out in triplicate. The *antifungal activity* was measured as the diameter (mm) of clear zone of growth inhibition. Thehumidity in incubation room should be maintained from 40 % to 50 % [10].

Immunomodulatory Activity: Luminol-enhanced chemiluminescence assay was performed as described by Helfand *et al.* [11]. Briefly, whole blood (diluted 1:200) neutrophils (1 x 107) and PMNs (1 x 106), were suspended in Hank's balance salt solution with calcium and magnesium ($HBSS^{++}$) and incubated with 50 uL of test compounds concentrations (1.6-50 ug/mL) for 30 min. To each well, 50 uL (20 mg/mL) zymosan (Sigma Chemical Co. USA), followed by the addition of 50 uL (7 x 10s M) luminol (G-9382 Sigma Chemical Co.) and then $HBSS^{++}$ were added to adjust the final volume to 0.2 mL. $HBSS^{++}$ was used as a control. Chemiluminescence's peaks were recorded with a Luminometer (Luminoskan RS Lab system, Finland).

Statistical Analysis: The data expressed are median inhibitory concentrations (IC_{50}) with ± standard error of mean.

RESULTS AND DISCUSSION

In vitro antifungal study was performed by subjecting different micro organisms to various fractions of *Nepeta suavis* in different solvents. Five fractions were used in the studies named aqueous, chloroform, hexane, butanol and ethyl acetate. Among these butanol and chloroform fractions showed significant antifungal activities. Diameter of zones of inhibition in mm of butanol against *Trichophyton longifusus*, *Aspergillus flavus Microsporum canis* and *Fusarium solani* were 24, 27, 20 and 16mm respectively. While chloroform fraction showed significant activity against four organisms, *Trichophyton longifusus, Aspergillus flavus, Candida albican* and *Fusarium solani* with a zone of inhibitions 22, 20, 18 and 17mm in diameter respectively, While ethyl acetate has no activity at all as shown in Table 1 (Fig 1). Concentration of sample 200 µg/ml of dimethyl sulfoxide (DMSO) and reference antifungal drugs miconazole and amphotericin B were served as negative and positive controls, respectively. The test tubes were incubated at 27-29 °C for 7-10 days. Growth in the medium containing the sample was determined by measuring linear growth (mm) and growth inhibition was calculated in % with reference to negative control [12,13].

The oxidative burst of polymorphoneutrophils (PMNs) and their ability to inhibit reactive oxygen species (ROS) were analyzed for the various fractions of *Nepeta suavis* including ethyl acetate, aqueous and chloroform fractions. Phagocytic cells on activation induce release of reactive oxygen free radicals (oxidative burst), which is then quantified by a luminol-enhanced chemiluminescence assay. A measurement of

Table 1: *In vitro* antifungal activity of *Nepeta suavis* various fractions and the reference standard, amphotericin B

	Diameter of zones of inhibition in mm				
	T. longifusus	*A. flavus*	*C. albicans*	*M. canis*	*F. solani*
Hexane	10±.5	8±.53	-	7±53	-
Chloroform	22±1.42	20±1.33	18±.15	-	17±.35
Ethyl acetate	-	-	-	-	-
Butanol	24±1	27±.5	-	20±.1	16±.15
Aqueous	11±.53	9±1		13±.1	
Amphotericin- B	28±1	30±1	33±1	29±1	32±1

Table 2: Immunomodulatory activity of *Nepeta suavis* various fractions and the control drug, Ibuprofen

Plant samples	with whole blood	with PMNs
Ethyl acetate	31.8 ± 5.9 µg/ml	53.5 ± 4.8 µg/ml
Chloroform	113.0 ± 2.6 µg/ml	15.8 ± 0.3 µg/ml
Aqueous	195 ± 1.5 µg/ml	197 ± 2.3 µg/ml
Ibuprofen	11.2 ± 1.9 µg/ml	2.88 µg/ml

PMNs = Polymorphoneutrophils

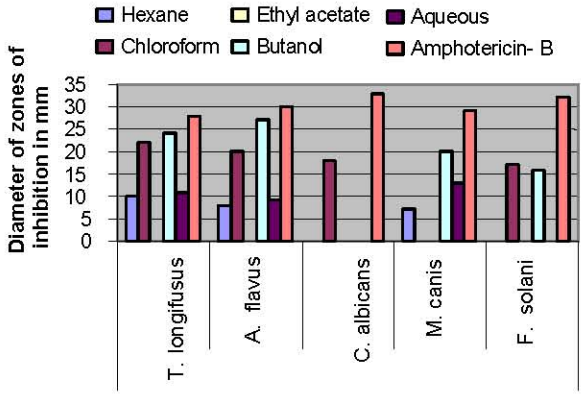

Fig. 1: *In vitro* antifungal activity of Nepeta suavis various fractions and the reference standard, amphotericin B

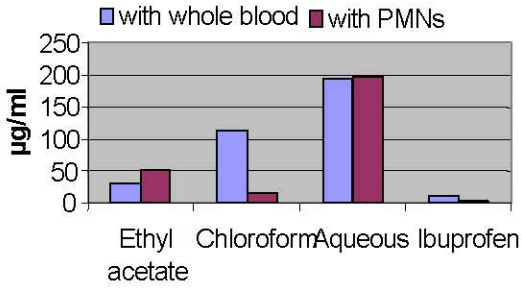

Fig. 2: Immunomodulatory activity of Nepeta various fractions the control drug,

chemiluminescence is an efficient and highly sensitive to investigate the different kinds of reactive oxygen species (HO, O^{-2} and H_2O_2). Luminol dependent chemiluminescence is a convenient method for detection of super oxide radicals anion in a biological system. Various concentrations of the crude extract of *Nepeta suavis* were incubated with PMNs for 30 minutes. After the addition of serum treated zymosan and luminol, phagocytic cells were scanned at 37°C for their chemiluminescence's activity. Ibuprofen was used as positive control. Ethyl acetate, aqueous and chloroform fractions from *Nepeta suavis* were screened over a wide range of concentration (6.25-200 µg/ml) for their possible modulatory effect on the oxidative burst in whole blood and PMNs, using a luminol based chemiluminescence assay [14]. The result of different assays employed in this study showed that chloroform fraction has a potential suppressive effect and clear inhibitory activity for oxidative burst of PMNs at a concentration of 15.8 µg /ml as compared to ethyl acetate and aqueous fraction, while ethyl acetate has a significant potential suppressive effect in whole blood at a concentration of 31.8 µg /ml in this assay as shown in the Table 2 (Fig. 2). This exhibited a clear suppressive effect on phagocytosis response upon activation with serum opsonized zymosan in a dose dependent manner. Proposed implications of the immunomodulatory activity are inhibitors for ROS inflammation control or other immunomodulatory uses.

CONCLUSION

In conclusion the results of the present study indicate that fractionated samples of *Nepeta suavis* possess significant antifungaland immunomodulatory activities. In order to further exploit the *in vivo* antifungal and immunomodulatory activities of this

indigenous medicinal plant and to come up with a potent, safe and economically affordable formulation, further investigations are to be required.

ACKNOWLEDGEMENTS

The authors wish to thank the Higher Education Commission (HEC), Government of Pakistan for providing financial support for the current study under the National Research Program for Universities (NRPU).

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