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INSECTICIDAL ACTIVITIES AND PHYTOCHEMICAL SCREENING OF CRUDE EXTRACTS AND ITS DERIVED FRACTIONS FROM THREE MEDICINAL PLANTS *NEPETA LEAVIGATA, NEPETAKURRAMENSIS* AND *RHYNCHOSIA RENIFORMIS*

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Abstract

The extracts and its derived fractions from three medicinal plants species *Nepeta leavigata*, *Nepeta kurramensis* and *Rhynchosia reniformis* were tested for insecticidal activities and preliminary phytochemical evaluation with the intention of standardization and proper manage of bioactive principles in such heterogonous botanicals and to encourage drug finding work with plants. The crude extracts and fractions from *Nepeta* plants showed moderate to strong insecticidal activity. Among the fractions from *Nepeta kurramensis* the *n*-butanol fraction showed strongest insecticidal activity with 89% mortality rate against *Tribolium castaneum* followed by methanol extract with 88% mortality ratio and in case of *Nepeta leavigata* the potential activity was showed by methanol extracts with 93% mortality rate against the tested insect. Surprisingly none of the extract / fractions obtained from *Rhynchosia reniformis* plant exhibited any insecticidal activity. The phytochemicals screening results revealed that both species of *Nepeta* showed similar phytochemicals profile. The group of chemicals terpenes, flavonoids and glycosides were observed in all the extracts/fractions of *Nepeta* plants. While phenolic compounds, acidic compounds and alkaloids were found in methanolic extracts, chloroform fraction and ethyl acetate fraction. The *Rhynchosia reniformis* was observed to be a good source of phenolic compounds, flavonoids, terpenes, alkaloids and fats.

Keywords: Medicinal plants, *Nepeta leavigata*, *Nepeta kurramensis*, *Rhynchosia reniformis*, Insecticidal activity, Biochemical screening, Drug sighting.

Introduction

Medicinal plants are the richest source of drugs by bringing new therapeutic agents. Our county has a rich store of phyto medicinal flora which is still undiscovered. Traditional medicinesare the economical source of therapy to population (Bashir *et al*., 2015). The extensive use of herbal medicines for healthcare measureshas been noted and several novel natural products with healing properties are commercialized (Muhammad *Riaz and Najm Ur Rahman***.** 2015). In reality, plants produce a diverse variety of bioactive molecules, making them an affluent resource of different types of medicines (Shinwari *et al.,* 2013). The medicinal properties of plants have been probe in the light of up-to-date scientific expansion all over the world, due to their heady pharmacological tricks and stumpy toxicity (Vaquero *et al*., 2010). Medicinal plants are extensively used in the cure of different diseases. Plant extracts and their diverse formulations in the healing and easing of several diseases in folk remedy have been dated back to the ancient times (Kamal et al. 2016). Crude extracts of medicinal plants can be subjected to chromatographic techniques for the isolation of natural products and to get valuable antibiotic, anticancer and herbicidal compounds (Zul *et al*., 2015).

Materials and Methods

The shade dried powderof *Nepeta leavigata* was soaked in methanol for 10 days. Thepowdered drug was extracted with 80 % methanol three times and filtered at room temperature. The filtrate was evaporated in rotary to get a dark-greenish residue (extract), which was further suspended in water and partitioned successively with *n*-hexane, chloroform, ethyl acetate and *n*-butanol to obtain *n*-hexane-soluble, chloroform-soluble, ethyl acetate-soluble, *n*-butanol-soluble and aqueous fractions, respectively. Same extraction and fractionation procedure was adopted for *Nepeta kurramensis* and*Rhynchosia reniformis*

In order to screen the lethal effects of the selected plants, the crude extracts and its derived fractions of *Nepeta leavigata*, *Nepeta kurramensis* and *Rhynchosia reniformis* were evaluated against insect *Tribolium castaneum*. To prepare the stock solutions 25 mg of each extracts were dissolved in 1ml DMSO and loaded in Petri dishes covered with the filter papers. To each Petri plate 10 test insects were added and incubated at 27°C for 24 hours with 50% relative humidity in growth chamber. The results were analyzed as percentage mortality, calculated with reference to the positive and negative controls. Permethrin was used as a standard drug, while and test insect was used as positive and negative controls (Rashid *et al*., 2009). Each experiment was repeated three times, and the number of flies survived per vial were counted in both control and test cultures. The percentage mortality was calculated by the formula:

$$\text{Growth regulation (\%)} = \frac{\text{Number of insects alive in test}}{\text{Number of insects dead in control}} \times 100$$

The phytochemicals screening of plants were performed as per well established protocols (Prabu *et al.*, 2009).

Results and Discussions

The insecticidal results revealed that crude extracts and fractions from both *Nepeta* species showed moderate to strong insecticidal activity which supports the traditional anti insect value (mosquito replant) of the *Nepeta* species. Among the fractions from *Nepeta kurramensis* the *n*-butanol fraction showed strongest insecticidal activity with 89% mortality rate against *Tribolium castaneum* followed by methanol extract with 88% and ethyl acetate fraction with 79% mortality rate and in case of *Nepeta leavigata* the potential activity was showed by methanol extracts with 93% mortality rate against the tested insect while rest of fractions showed above 80% mortality with the exception of water fraction which exhibited 52% mortality. Surprisingly none of the extract /fractions obtained from *Rhynchosia reniformis* plant exhibited any insecticidal activity (Table 1). Safe storage of grains and food products against insect damage is a serious concern (Haq *et al*., 2005). Therefore an eco-friendly approach (Upadhyay & Jaiswal, 2007) is always encouraged. Some plant extracts are highly effective and safe for human beings and environment, convenience and inexpensive for protection of stored grains (Hasan *et al*., 2006). The use of insecticides causes several problems such as environmental pollution, health hazards, pesticide resistance and outbreak of pests due to disrupt biological control and ecosystem (Shah *et al*., 2008). To compact with these problems, there is an urgent need, alternative and safe effective methods with no toxic effects on non-target organisms. This has created interest in research of using plant extracts as alternative methods to control pests (Boussaada *et al*., 2008). Many plants like *Annona squamosa* (L.), *Lantana camara, Clerodendrun inerme, Cassia fistula, Azadirachta indica* and *Calotropis procera*are proved to be lethal to various stored grain pests and delay the developmental stages by interfering with their apolytic and molting processes (Deka & Singh, 2005).

It is well known that the chemical composition in plant species may differ significantly within the same taxon, depending on genetic and geographical parameters (e.g., climatic, seasonal) and their toxic effects may be species-specific (Nadeem et al. 2013). In addition to their insecticidal activity, it was found that *Nepeta* speciespresent strong antimicrobial (Shinwari *et al*., 2013) and antileshmanial activities (Almas *et al.,* 2012). Both the species of *Nepeta* plant showed similar phytochemical profile. *Nepeta* species were found to be rich source of preliminary phytochemicals. Flavonoids, terpenes and glycosides were found in all the extracts/fractions of both species while phenolic compounds, acidic compounds and alkaloids were reported in methanol extract and chloroform/ethyl acetate fraction of both *Nepeta* species (Table 2). The biological activities of medicinal plants are due to presence of phytochemicals and there is a strong correlation between biological potential of a plant and its metabolites. In case of *Rhynchosia reniformis* most of the phytochemicals were reported from methanol extract, *n*-hexane fraction and chloroform fraction (Table 3). The phytochemicals detected in our extracts are well known for various pharmacological activities. For example alkaloids are common antibacterial, antimalarial, cytotoxic and anticancerous agents (Wirasathien *et al.,* 2006). Similarly saponins have the insecticidal, antibiotic, fungicidal properties (Sparg *et al.,* 2004). Flavonoids have been shown to have antibacterial, anti-inflamatory, antiallergic, antineoplastic, antiviral, anti-thrombotic antioxidant and vasodilatory activities (Miller, 1996).

Table 1. Insecticidal activities of crude extracts/fractions from *Nepeta laevigata, Nepeta kurramensis* and *Rhynchosia reniformis* (Conc. 0.25 mg/ml).

Plants	Methanol extract	*n*-hexane fraction	Chloroform fraction	Ethyl acetate	*n*-butanol fraction	Water fraction	DMSO	Drug
N. leavigata	88% SE+0.000	71% SE+0.000	73% SE+0.000	79% SE+0.000	89% SE+0.000	64% SE+0.000	000% SE+0.000	100% SE+0.000
N.kurramensisi	93% SE+0.000	80% SE+0.000	81% SE+0.000	80% SE+0.000	81% SE+0.000	52% SE+0.000	000% SE+0.000	100% SE+0.000
R. reniformis	00% SE+0.000	00% SE+0.000	00% SE+0.000	00% SE+0.000	00% SE+0.000	00% SE+0.000	000% SE+0.000	100% SE+0.000

Table 2. Preliminary phytochemical studies of *Nepeta kurramensis* and *Nepeta laevigata*.

S. No.	Phytochemical tests	Methanol extract	*n*-hexane fraction	Chloroform fraction	Ethyl acetate fraction	*n*-butanol fraction	Water fraction
1.	Phenolic compound	+	-	+	+	-	-
2.	terpenes	+	+	+	+	+	+
3.	Flavonoids	+	+	+	+	+	+
4.	Alkaloid	+	-	+	+	-	+
5.	Carbohydrates	+	-	-	-	-	+
6.	Glycosides	+	+	+	+	+	+
7.	Resin	-	-	-	-	-	-
8.	fats	-	-	+	+	-	-
9.	Acidic compounds	+	-	+	+	-	-
10.	Mucilage	-	-	-	-	-	-
11.	Proteins	+	-	-	-	-	+

Present = +, Absent = -

Table 3. Preliminary phytochemical studies of *Rhynchosia reniformis*.

S. No.	Phytochemical tests	Methanol extract	*n*-hexane fraction	Chloroform fraction	Ethyl acetate fraction	*n*-butanol fraction	Water fraction
1.	Phenolic compound	+	+	+	-	-	-
2.	terpenes	+	+	+	+	+	+
3.	Flavonoids	+	-	+	+	-	+
4.	Alkaloid	+	-	-	+	-	+
5.	Carbohydrates	+	-	+	-	-	+
6.	Glycosides	+	-	-	+	-	+
7.	Resin	-	-	-	-	-	-
8.	fats	+	+	+	-	-	-
9.	Acidic compounds	+	-	-	-	-	-
10.	Mucilage	-	-	-	-	-	-
11.	Proteins	-	-	-	-	-	-

Present = +, Absent = -

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