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ANTIMICROBIAL EVALUATION AND PROXIMATE PROFILE OF *NEPETA LEAVIGATA*, *NEPETA KURRAMENSIS* AND *RHYNCHOSIA RENIFORMIS*

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Abstract

The antimicrobial screening of the crude extracts and solvent soluble fractions of *Nepeta leavigata*, *Nepeta kurramensis* (*Lamiaceae*) and *Rhynchosia reniformis* (*Papilionaceae*) were investigated along with the proximate profile of the plants for the purpose of standardization and quality control of bioactive components in such heterogonous botanicals and aid to drug discovery work with botanicals. The antibacterial results of *Nepeta leavigata* showed that the *n*-butanol fraction displayed significant activity (85% inhibition) against *Escherichia coli* and *Proteus morganii* (83% inhibition), while in *Nepeta kurramensis* chloroform fraction exhibited promising activity (89% inhibition) against *Streptococcus cricetus*, and *Micrococcus flavas* (84% inhibition). In *Rhynchosia reniformis*, only crude extract exhibited 100% inhibition against *Streptococcus cricitus* while ethyl acetate fraction showed (99% inhibition) against *Micrococcus flavas*, *Streptococcus cricitus* (95% inhibition), and *Proteus morganii* (90% inhibition). In antifungal activities; chloroform and ethyl acetate fractions of *Nepeta leavigata* as well as chloroform fraction of *Nepeta kurramensis* were promising; while in *Rhynchosia reniformis* chloroform, n-hexane and methanolic extracts were significant inhibitors as compared to rest of fractions. In this study the proximate composition of these medicinal plants was also assessed and the analysis was carried according to AOAC methods. All the selected species were found to be a good source of ash, proteins and fats which can contribute greatly towards nutritional requirements and adequate protection against microorganism and other diseases.

Introduction

Plants are important source of natural drugs and medicinal plants have been in use for the eradication of human sufferings since ancient times (Gul *et al*., 2012). Today, many scientists and medical experts around the world are emphasizing the value of herbal remedies for health (Ali & Azhar, 2000). The genus *Nepeta* (also known as *Glechoma* & *Cataria*) is named after the ancient Italian City of Nephi. Among these 67 species of the genus *Nepeta* are found in Iran while 58 species have been found in Pakistan. *Nepeta* species are used as diuretic, diaphoretic, vulnerary, antispasmodic, antiasthmatic, tonic, febrifuge and sedative agents (Hussain *et al*., 2008). Some of the Iranian *Nepeta* species are employed in Iranian folk and traditional medicine where they find good use in the treatment of various disorders, such as nervous, respiratory and gastrointestinal diseases (Hussain *et al*., 2010). Similarly *Rhynchosia* species are used as antinociceptive and anti-inflammatory agents and some species also have anti-fertility and antipyretic effects (Yim *et al*., 2009; Wang *et al*., 2007; Vimala *et al*., 1997). *N. leavigata* and *N. kurramensis* are perennial herbs, stems erect, 30-80 cm, usually branched; flowering period is June to August (Nasir, 1990). *R. reniformis* belong to family *Papilionaceae*, are found mainly in sandy soil and grows to about 25 cm tall and is easy to identify because of its orbicular to reniform leaves, yellow flowers, and habitat (Nasir & Ali, 1977).

The medicinal importance of genus *Nepeta* and *Rhynchosia* prompted us to investigate antimicrobial activities and proximate analysis of *N. laevigata*, *N. kurramensis* and *R. reniformis* in current study. Up to our best knowledge no proximate work has been done on investigated species.

Materials and Methods

Plant material: *N. leavigata* and *N. kurramensis* were collected in July 2009 from Swat and Kurram Agency and *R. reniformis* was collected from Karak, Khyber Pakhtunkhwa, Pakistan. The taxonomic identity of these plants was determined by plant taxonomist at Department of Plant Science, Kohat University of Science and Technology, Kohat and the voucher specimens have been kept for future reference with voucher no. PLS/Herb 1015, PLS/Herb 1016 and PLS/Herb 1017. After collection, the selected species were washed under running tap water, air dried, homogenized to fine powder and stored in airtight bottles at room temperature.

Extraction and fractionation: The shade-dried whole plant material of *N. leavigata* (7 kg), *N. kurramensis* (7.5 kg) and *R. reniformis* (10 kg) was chopped and soaked in methanol for 3 weeks and filtered. The filtrate was evaporated under reduce pressure through Rota vapor to get dark-greenish extract, which was further suspended in water and partitioned successively into *n*-hexane, chloroform, ethyl acetate and *n*-butanol fractions. The crude plant extracts and subsequent fractions were subjected to microbial bioassay.

Evaluation of bioassays

Antibacterial assay: The bacterial strains studied were *Bacillus subtilis, Pseudomonas testosteroni, Eschericha coli, Klebsiella pneumonia, Streptococcus cricetus, Proteus morganii*, and *Micrococcus flavus.* Loop full of the strain was inoculated in 30mL of nutrient broth in a conical flask and is incubated on a rotary shaker for 24h to activate the strain. Mueller Hinton Agar No. 2 was prepared for the

study. The assay was performed using two methods. Agar disk diffusion (Salie *et al.*, 1996; Rahman & Rashid, 2008) for the aqueous extract and Agar ditch diffusion (Perez *et al.*, 1990) for solvent extract. The media and the test bacterial cultures were poured into Petri dishes (Hi-Media). The test strain (0.2mL) were inoculated into the media (inoculum's size 108 cells/mL) when the temperature reached 40-42°C. Care was taken to ensure proper homogenization. The experiments were performed under strict aseptic conditions.

For the Agar disk diffusion method, the test fraction was introduced onto the disk (0.7cm) (Hi-Media) and then was allowed to dry. Thus the disk was completely saturated with the test fraction. Then the disks were introduced onto the upper layer of the medium with the bacteria. The plates were incubated overnight at 37°C. For the Agar ditch diffusion method, after the medium was solidified, a ditch was made in the plates with the help of a cup-borer (0.85cm). The test fractions were introduced into the well and the plates were incubated overnight at 37°C. Microbial growths were determined by measuring the diameter of the zone of inhibition. Methanol and distilled water were used as the control. The control activity was deducted from the test and the results obtained were plotted (Perez *et al.*, 1990).

Antifungal bioassay: Agar Tube Dilution Method was used for antifungal bioassay (Choudhary *et al*., 1995). The crude extracts and solvents soluble fractions were dissolved in DMSO (24µg/mL) and Sterile Sabourd Dextose Agar was used as a standard drug. The sample solution was further diluted to 1:10 with medium prior to test. The samples (extracts and fractions) were subjected to antifungal activity assays against *Aspergillus niger, Aspergilus fumigatus, Aspergilus flavus, Fusarium solani, Candida albicans, Rhizoctonia solani, Pseudallescheria boydii and Microsporum canis*. The minimum inhibitory concentration (MIC) was determined for each test sample.

Proximate analysis: Fresh powdered plant materials of *Nepeta leavigata, Nepeta kurramensis,* and *Rhynchosia reniformis* (*Papilionaceae*) were further subjected to proximate analysis. Proximate analysis determines moisture, ash, proteins, fats, fibers, carbohydrates and energy value reported as the percentage composition of the product. AOAC methods were applied to carry out proximate analysis of the fresh plant samples. The moisture (3g sample for each analysis) was determined by a manual drying method in a WiseVen oven (WOF-105, Version 1.4.3) at 105°C for 6 hrs (Anon., 2003), and ash value was obtained by dry ashing in Wise Therm muffle furnace (FP-03, Version 1.4.4) at 550°C for 3 hrs. Crude lipid was quantified using the soxhlet apparatus and *n*-hexane as a solvent by the reported method (Anon., 2003). Crude fiber was estimated by acid-base digestion with 1.25% H_2SO_4 (v/v) and 1.25% NaOH (w/v) solutions (Hussain *et al.*, 2010). The nitrogen value was determined by micro Kjeldahl method described by Pearson (1976) and the nitrogen content was converted to protein by multiplying with factor of 6.25. Carbohydrate content was determined by difference, while energy value was calculated by multiplying the amount of proteins and carbohydrates by a factor of 4 and lipid by the factor of 9 K cal/100g. All parameters were done in triplicates and reported in percentage (Anon., 2000; Anon., 2003).

Statistical analysis: Each experiment was repeated three times. The results are presented with their means, standard deviation and standard deviation using Microsoft Office Excel 2003.

Results and Discussion

Evaluation of Bioassays: Plants have been used since ancient times for the treatment of various diseases from flu to cancer (Sarwat *et al*., 2012). Approximately two-thirds to three-quarters of the world's population rely on medicinal flora as their main source of medicines (Qayum *et al*., 2012). The crude extract and fractions of *N. leavigata, N. kurramensis* and *R. reniformis* were screened against various human pathogens and the results are shown in Tables.

The antibacterial results of *N. leavigata* showed that the *n*-butanol fraction displayed promising activity against *E. coli* (85% inhibition), *P. morganii* (83% inhibition) and moderately active against M. *flavas* (73% inhibition), and *B. subtilis* (70% inhibition). However it was inactive against *P. testosteroni* and *K. pneumoniae*. The *n*-hexane fraction showed low activity against all pathogens (Table 1). In case of *N. kurramensis*, chloroform fraction was significantly active against *Streptococcus cricetus* (89% inhibition), and *Micrococcus flavas* (84% inhibition) and was active against *P. testosteroni* (71% inhibition) (Table 2), Where in *R. reniformis* crude extract exhibited 100% inhibition against *Streptococcus cricitus* and ethyl acetate fraction showed promising activity against *M. flavas* (99% inhibition), *S. cricitus* (95% inhibition) and *P. morganii* (90% inhibition) (Table 3).

In this study, antifungal activity of the crude extract and various fractions of the plants under investigation were also evaluated against fungal pathogens and the results are shown in Tables. The chloroform and ethyl acetate fractions of *N. leavigata* were active against *A. fumigatus, F. solanim, C. albicans, R. solani,* and *M. canis* (Table 4) and chloroform fraction of *N. kurramensis* showed inhibition against *A. fumigatus, P. boydii,* and *C. albicans* (Table 5), while in *R. reniformis*, chloroform, n-hexane and methanolic extracts were more reactive against most pathogens as compared to rest of fractions (Table 6).

Since synthetic drugs are quite expensive and have many side effects therefore the development of new effective and safe products for the treatment of different diseases caused by human pathogens is highly desirable (Christopher, 2004; Fiona & John, 2004). The plants under investigation showed significant antimicrobial activities which support the traditional use of these plants against various diseases. Similar studies were conducted on other indigenous species (Walter *et al*., 2011; Shinwari *et al*., 2009). Therefore these plant species could be an excellent natural source for the treatment of diseases and might be potent targets for the activity guided isolation of its active constituents. In order to explore the mechanisms of action and potentially relevant uses, further work is in progress for isolation and identification of bioactive constituents.

Table 1. Antibacterial activity of crude extract and various fractions of *N. laevigata*.

Name of bacteria	Crude extract zone of inhibition (mm)	Inhibition percentage	n-Hexane fraction Zone of inhibition (mm)	Inhibition percentage	Ethyl acetate fraction Zone of inhibition (mm)	Inhibition percentage	Chloroform fraction Zone of inhibition (mm)	Inhibition percentage	n-butanol fraction zone of inhibition (mm)	Inhibition percentage	Water fraction Zone of inhibition (mm)	Inhibition percentage
B. subtilis	Nil	Nil	6	25	10	32	Nil	Nil	18	**70**	Nil	Nil
P. testosterone	8	29	11	36	11	36	Nil	Nil	Nil	Nil	3	15
E. coli	7	27	7	27	Nil	Nil	6	25	20	**85**	Nil	Nil
K. pneumoniae	8	29	6	25	Nil	Nil	Nil	Nil	Nil	Nil	7	27
S. cricetus	Nil	Nil	9	30	Nil	Nil	6	25	12	40	Nil	Nil
P. morganii	5	23	5	23	Nil	Nil	7	27	19	**83**	Nil	Nil
M. flavas	Nil	Nil	6	25	Nil	Nil	9	30	18	**73**	Nil	Nil
Antibiotic agent	*K.pneumoniae*	63	*B. subtilis*	83	*E. coli*	76	*P.testosteroni*	65	S. C	81	*M. flavas*	39
Nulidixic acid	15		19		18		16		19		13	
Antibiotic agent	K.*pneumoniae*	67	*B. subtilis*	76	*E. coli*	65	*P.testosteroni*	67	S. C	99	*M. flavas*	67
dixoycline	17		18		16		17		24		17	

The plates were inoculated at a concentration of 3 mg/mL of DMSO

Table 2. Antibacterial activity of crude extract and various fractions of *N. kurramensis*.

Name of bacteria	Crude extract zone of inhibition (mm)	Inhibition percentage	n-Hexane fraction Zone of inhibition (mm)	Inhibition percentage	Ethyl acetate fraction Zone of inhibition (mm)	Inhibition percentage	Chloroform fraction Zone of inhibition (mm)	Inhibition percentage	n-butanol fraction zone of inhibition (mm)	Inhibition percentage	Water fraction Zone of inhibition (mm)	Inhibition percentage
B. subtilis	8	29	Nil	Nil	6	25	11	36	9	30	Nil	Nil
P. testosterone	Nil	Nil	Nil	Nil	6	25	17	**71**	5	23	Nil	Nil
E. coli	Nil	Nil	7	27	Nil	Nil	8	29	14	60	6	20
K. pneumoniae	4	16	Nil	Nil	5	23	10	32	Nil	Nil	Nil	Nil
S. cricetus	Nil	Nil	7	27	12	40	20	**89**	7	27	Nil	Nil
P. morganii	Nil	Nil	Nil	Nil	8	29	11	36	6	25	4	16
M. flavas	4	16	Nil	Nil	6	25	19	**84**	5	23	Nil	Nil
Antibiotic agent	*K.pneumoniae*	90	*B. subtilis*	70	*E. coli*	95	*P.testosteroni*	70	S. C	90	*M. flavas*	65
Nulidixic acid	20		18		22		18		20		16	
Antibiotic agent	*K.pneumoniae*	70	*B. subtilis*	70	*E. coli*	67	*P.testosteroni*	71	S. C	95	*M. flavas*	63
dixoycline	18		18		17		19		22		15	

The plates were inoculated at a concentration of 3 mg/mL of DMSO

Table 3. Antibacterial activity of crude extract and various fractions of *R. reniformis*.

Name of bacteria	Crude extract zone of inhibition (mm)	Inhibition percentage	n-Hexane fraction Zone of inhibition (mm)	Inhibition percentage	Ethyl acetate fraction Zone of inhibition (mm)	Inhibition percentage	Chloroform fraction Zone of inhibition (mm)	Inhibition percentage	n-butanol fraction zone of inhibition (mm)	Inhibition percentage	Water fraction Zone of inhibition (mm)	Inhibition percentage
B. subtilis	15	63	12	40	9	30	17	52	13	38	6	25
P. testosterone	11	36	15	63	12	35	14	60	10	32	Nil	Nil
E. coli	9	30	8	29	18	**70**	Nil	Nil	Nil	Nil	5	23
K. pneumoniae	Nil	Nil	Nil	Nil	7	27	6	25	Nil	Nil	Nil	Nil
S. cricetus	25	**100**	Nil	Nil	22	**95**	20	**90**	Nil	Nil	Nil	Nil
P. morganii	Nil	Nil	5	23	20	**90**	12	40	Nil	Nil	Nil	Nil
M. flavas	Nil	Nil	7	27	24	**99**	10	32	6	25	Nil	Nil
Antibiotic agent	*K.pneumoniae*	70	*B. subtilis*	67	*E. coli*	71	*P.testosteroni*	71	S. C	70	*M. flavas*	63
Nulidixic acid	18		17		19		19		18		15	
Antibiotic agent	*K.pneumoniae*	67	*B. subtilis*	71	*E. coli*	90	*P.testosteroni*	71	S. C	71	*M. flavas*	38
dixoycline	17		19		20		19		19		13	

The plates were inoculated at a concentration of 3 mg/mL of DMSO

Table 4. Antifungal activities of crude extract and various fractions of *N. leavigata* (MIC* (mg/mL)).

Fungal strain	Methanol extract	*n*-Hexane fraction	Chloroform fraction	Ethyl acetate fraction	*n*–Butanol fraction	Aqueous fraction
A. niger	3	2.5	2	NA	NA	3
A. fumigatus	NA	2	3	3	2	2
A. flavus	2	3	2	2	NA	3
F. solani	2	3	3	2	2.5	NA
C. albicans	NA	NA	4	3	NA	NA
R. solani	2	NA	3	3	2	NA
P. boydii	NA	2	2	2.5	NA	NA
M. canis	NA	NA	3.5	3	NA	NA

MIC*= Minimum inhibitory concentration
NA* = Not active
The concentration of relent extract used was 24 mg/mL of DMSO

Table 5. Antifungal activities of crude extract and various fractions of *N. kurramensis* (MIC* (mg/mL)).

Fungal strain	Methanolic extract	*n*-Hexane fraction	Chloroform fraction	Ethyl acetate fraction	*n*–Butanol fraction	Aqueous fraction
A. niger	NA	NA	2.5	NA	2	NA
A. fumigatus	NA	2.5	4	2	NA	NA
A. flavus	NA	NA	2	NA	NA	NA
F. solani	2	NA	2.5	2	NA	NA
C. albicans	NA	NA	3	2	NA	2.5
R. solani	NA	NA	2.5	NA	NA	NA
P. boydii	NA	2	4	NA	2	NA
M. canis	NA	NA	3.5	NA	NA	2

MIC* = Minimum inhibitory concentration
NA* = Not active
The concentration of relent extract used was 24 mg/mL of DMSO

Table 6. Antifungal activities of crude extract and various fractions of *R. reniformis* (MIC* (mg/mL)).

Fungal strain	Methanolic extract	*n*-Hexane fraction	Chloroform fraction	Ethyl acetate fraction	*n*–Butanol fraction	Aqueous fraction
A. niger	3	2	2	NA	3	3
A. fumigatus	2	3.5	2	3	2	2
A. flavus	3.5	2	2.5	2	NA	NA
F. solani	2.5	3	2	2.5	NA	NA
C. albicans	3	4	NA	NA	NA	NA
R. solani	3	3.5	NA	2	2	NA
P. boydii	2	3.5	2	NA	NA	NA
M. canis	2	3	2.5	NA	NA	NA

MIC* = Minimum inhibitory concentration

NA* = Not active

The concentration of relent extract used was 24 mg/mL of DMSO

Proximate analysis: The results of the proximate analysis of the plant species with their standard deviation are summarized in Table 7. The moisture content was found to be highest in *N. kurramensis* (3.53%), whereas *R. reniformis* was found to be the lowest (2.35%). As a result of this study, *N. kurramensis* was found to contain the highest content of the moisture. The descending order of the moisture content was observed as *N. kurramensis* > *N. laevigata* > *R. reniformis*. The moisture content of plant species were found lower than *N. sauvis* (8.44%) reported by Hussain *et al*, (2011). The highest ash and protein value were observed in *N. laevigata* during analysis. Ash contents of *N. laevigata*, *N. kurramensis* and *R. reniformis* were found to have 18.55, 35.15 and 11.95% respectively.

The results of ash content with standard deviation are presented in Table 7. In comparison, ash content was found higher than the *N. sauvis* (7.91%) (Hussin *et al*., 2011). The ash content of *N. kurramensis* was found similar to that of *Datura alba* (18.80%), *Phlomis cashmeriana* (17.66%) and *Calotropis procera* (17.62%), while ash values of *R. reniformis* was also in good agreement with *Dalbergia sisso* (12.33%), *Phlomis bracteosa* (10.83%), and slightly lower than *Avera javanica* (14.23%) (Hussain *et al*., 2011; Hussain *et al*., 2010).

N. kurramensis was found to be highest in its crude fat content (8.27%). However, *R. reniformis* contains crude fat (1.79%) similar to that of *Aerva javanica* having 1.15% fat (Hussain *et al*., 2011). *N. laevigata* were found to have 3.72% fat and showed close similarity with *Rhiza stricta* (3.98%) and *Dalbergia sisso* (3.35%) (Hussain *et al*., 2010). *Nepeta* species were found to be a good source of fats particularly *N. kurramensis* (Table 7).

Crude fiber of *R. reniformis* was found to have value of 31.59%, followed by the *N. kurramensis* (10.326%) (Table 7). In comparison with other medicinal plants *R. reniformis* showed close resemblance with *Aerva javanica* (29.186%) and *Calotropis procera* (29.49%) (Hussain *et al*., 2010). A high intake of dietary fiber improves glycemic control, decreases hyperinsulinemia, and lowers plasma lipid concentrations (Chandalia *et al*., 2000). Therefore, dietary guidelines for patients with diabetes should emphasize an overall increase in dietary fiber through the consumption of unfortified sources, rather than the use of fiber supplements.

The protein content of the medicinal plants was calculated on the basis of the available nitrogen using Kjeldahl method and was observed in the range of 0.44-10.22% with *N. laevigata* having the highest value (10.22%), followed by *N. kurramensis* (6.31%) and *R. reniformis* (0.44%) (Table 7). The *N. laevigata* showed very close value to *Phlomis bracteosa* (10.61%) and *Phlomis cashmeriana* (9.51%) belong to the family Labiateae (Hussain *et at*., 2010).

The carbohydrate content of analyzed samples revealed that *N. kurramensis* had highest amount of carbohydrates (53.02%), followed by the decreasing order of *R. reniformis* (51.88%) and *N. laevigata* (42.52%) (Table 7). The contribution of the carbohydrates to the energy in a food ration recommended by Anon., (1990) is from 55 to 75%. The carbohydrates of *N. kurramensis* and *R. reniformis* fall in the acceptable range set by WHO. Thus these 2 plant species can be used as a source of energy contribution in a food ration.

According to the results of the energy calculations, based on the carbohydrates, fats, and protein content, the highest value was found in the *N. kurramensis* (311.73 kcal/100g), while the *R. reniformis* was found to contain the lowest energy value (225.48 kcal/100g) (Table 7). The energy value of the *Nepeta* species were found comparatively close to the reported values of some Nigerian leafy vegetables (248.8-307.1 kcal/100g) (Isong *et al*., 1999), some Ghanian green leafy vegetables like *Corchorus tridens* (283.1 kcal/100g) and sweet potato leaves (288.3 kcal/100g) (Asibey & Tavie, 1999). However, the energy value of *N. kurramensis* was observed to have similar value to that of *Calotropis procera* (312.41 kcal/100g) and *Datura alba* (308.10 kcal/100 g) reported by Hussain *et al*. (2011).

The correlation analysis of the selected parameters showed that similar parameters have highly significant correlation while among other parameters the correlation is either significant or non-significant, and in some cases moderate. Moisture with fats and energy value; ash with proteins; fats with energy value showed significant correlation. Ash with fibers and carbohydrates; fibers with proteins and proteins with carbohydrates displayed non-significant correlation (Table 8).

Table 7. Proximate values of the selected medicinal plants with standard deviation (%).

Species name	Moisture	Ash	Fats	Fibers	Proteins	Carbohydrate	Energy value
R. reniformis	2.35 ± 0.14	11.95 ± 0.17	1.79 ± 0.02	31.59 ± 0.89	0.44 ± 0.16	51.88 ± 0.81	225.48 ± 3.14
N. laevigata	2.63 ± 1.10	35.15 ± 0.38	3.72 ± 0.33	5.77 ± 0.73	10.22 ± 0.81	42.52 ± 2.08	244.39 ± 5.44
N. kurramensis	3.53 ± 0.68	18.55 ± 0.89	8.27 ± 0.42	10.33 ± 0.88	6.31 ± 0.17	53.02 ± 0.85	311.73 ± 6.19

Table 8. Correlation matrix of proximate parameters.

Parameters	Moisture	Ash	Fats	Fibers	Proteins	CHO	E.V
Moisture	1						
Ash	-0.02	1					
Fats	0.99	0.05	1				
Fibers	-0.55	-0.82	-0.60	1			
Proteins	0.33	0.94	0.40	-0.97	1		
Carbohydrates	0.39	-0.93	0.32	0.56	-0.74	1	
Energy value	0.99	-0.04	0.99	-0.54	0.32	0.40	1

CHO = Carbohydrates, E.V = Energy value

Conclusion

The results gleaned from this study showed that n-butanol fraction of *N. leavigata* exhibited excellent activity against *E. coli* and *P. morganii*, while chloroform fraction of *N. kurramensis* displayed significant inhibition against *S. cricetus* and *M. flavas*. The crude extract of *R. reniformis* showed 100% inhibition. Ethyl acetate fraction of *R. reniformis* also exhibited promising activity against *M. flava*, *S. cricetus* and *P. morganii*. The chloroform and ethyl acetate fractions of *N. leavigata* also exhibited promising antifungal activity, while chloroform fraction of *N. kurramensis* showed moderate activity against all fungal strains. In case of *R. reniformis*; chloroform, n-hexane and methanolic extract were more active as compared to other fractions. Results of proximate analysis revealed that all the selected species are a good source of ash, proteins and fats and can contribute greatly towards nutritional requirements.

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