PharmacologyOnLine
April 30, 2015
Archives • 2015 • vol.1 • 13-18

PHARMACOLOGICAL EVALUATION OF SAPONINS, METHANOLIC EXTRACT AND SUBSEQUENT FRACTIONS OF *RUMEX HASTATUS* D. DON AGAINST *MONOMORIUM PHARAONIS* AND *HETEROTERMES INDICOLA*

Ahmad, S.; Ullah, F.; Ayaz, M.*; Sadiq, A.; Zeb, A.; Imran, M.

Department of Pharmacy, University of Malakand, Dir (L), Pakistan

*Ayazuop@gmail.com

Abstract

Due to the biodegradable and non-hazardous nature of majority of natural bioactive compounds, the current study was designed to evaluate crude saponins (Rh.Sp), crude methanolic extract (Rh.Cr) and the resultant fractions (n-hexane (Rh.Hex), chloroform (Rh.Chf), ethyl acetate (Rh.EtAc), aqueous (Rh.Aq)) of *Rumex hastatus* for insecticidal activity against *Monomorium pharaonis* (Pharaoh ant) and *Heterotermes indicola* (termites). Extraction was carried out using cold maceration process and the Rh.Cr was fractionated into subsequent fractions using successive solvent-solvent extraction process. Similarly the crude saponins were isolated from powdered sample of *R. hastatus* using standard protocol. Furthermore, the direct contact toxicity method was followed to evaluate various samples of *R. hastatus* for insecticidal assay.

The Rh.Sp and Rh.Chf exhibited excellent insecticidal activities. In the assay against termites, the Rh.Sp, Rh.Cr, Rh.Hex, Rh.Chf, Rh.EtAc and Rh.Aq exhibited 100.0 ± 0.00, 49.32 ± 2.28, 56.00 ± 4.00, 93.32 ± 2.28, 80.00 ± 0.00 and 46.64 ± 6.12% lethality respectively on the first day. Similarly almost all the termites were killed by all the samples on the third day. In anti-pharaoh ant activity, the result of Rh.Sp was recorded as the highest. The LC_{50} of Rh.Sp, Rh.Cr, Rh.Hex, Rh.Chf, Rh.EtAc and Rh.Aq against pharaoh ant were recorded as <0.01, 3.2, 4.0, <0.1, 1.6 and 3.4 mg/ml respectively.

Based on the remarkable insecticidal activities of Rh.Sp, Rh.Chf and Rh.EtAc it is concluded that Rh.Sp, Rh.Chf and Rh.EtAc of *R. hastatus* may be a good source of insecticidal compounds, so these fractions should be subjected to isolation, purification and characterization which may lead to novel, safe and efficacious insecticidal drug development.

Key words: Saponins, *Rumex hastatus*, termites, Pharaoh ants, insecticide, lethality

Introduction

Human beings are most social animals on the earth [1]. The man wants to live in such an environment where there is proper storage condition for the goods and food stuffs. An environment where there is no chance of nuisance regarding the pests and reptiles. At every home there are numerous materials made of wood especially the doors, windows, boxes, furniture, cots, ceiling woods, tables and many more [2]. These goods are the daily needs of human beings but certain environmental factors don't allow the man to utilize these goods eternally. One of the basic factors is the nuisance and destruction of pests and the most prominent pest, which causes the destruction of wood, is the termite *Heterotermes indicola.* Another nuisance causing insect which is found everywhere is the pharaoh ant (*Monorium pharaonis*) [3]. The pharaoh ant has the property of detecting the foodstuffs though hidden in several boxes. These ants do not let the man to store the food materials without being fully protected . To get rid of these insects a lot of insecticides are in use from several decades [4].

Various chemical compounds are in use in the form of insecticides to protect wooden material, food substances, crops, paper material, leather materials and other valuable goods from various types of insects i.e., Horntails, Horsehair Worms, Millipedes, Pantry Pests, Termites, Wood-Boring Beetles in Homes, Wood Wasps, silverfish, earwigs, fleas, Cockroaches, clothes moths, ants, Conenose Bugs etc [5]. Moreover different types of chemicals used as insecticides are hazardous to human health in different ways [6]. But as the plant material contain various types of compounds which are mostly degradable and are generally not toxic to human health therefore natural products attract the attention of multiple investigators [7]. Various plants are scientifically verified to have insecticidal properties, for example; *Cymbopogon winterinus* Jewitti [8], *Chromolaena odonata* L. [9], and nutgrass tuber *Cyperus rotandus* L.[10].

Rumex is the genus of polygonaceae and several species of this genus are ethnobotanically used as insecticide [11]. Researchers are trying to isolate natural insecticidal compounds from various plants. Among various plant secondary metabolites, the saponins are the groups of compounds having insecticidal and other toxicological properties [12-14]. Based on the literature survey the current study is designed to evaluate the anti-pharaoh ant and anti antitermite activity of various extracts of *Rumex hastatus*.

Methods

Plant collection and extraction

The whole plant of *Rumex hastatus* was collected from the hilly area nearby University of Malakand, Chakdara (Lower dir) Khyber Pukhtunkhwa. The plant specimen was pressed and deposited with voucher number (1015SJ) in the herbarium, Department of Botany, Shaheed Benazir Bhuto University, Sheringal Dir Upper, KPK, Pakistan. The plant material was cleaned with water until all the unwanted particles (sands and dust particle) were removed. The plant material was spread on neat papers in a room protected from sunlight and completely shade dried for 20 days. After shade drying the plant material was converted into coarse powder using a cutter mill. The pulverized drug weighing almost 5 kg was dipped in 20 liters of 80% methanol. After soaking for 15 days the plant material was filtered and the filtrate obtained was concentrated by rotary evaporator under reduced pressure and temperature (40°C). The methanolic extract in concentrated form was solidified using water bath. The Rh.Cr obtained was 350 grams [15].

Fractionation

The fractionation was conducted using *n*-hexane, chloroform, ethyl acetate and distilled water by successive solvent-solvent extraction using separating funnel. This process was conducted by starting from low polar solvent towards the high polar solvent i.e., n-hexane < chloroform < ethyl acetate < distilled water. The suspension of Rh.Cr was made by adding 500 mL of distilled water into Rh.Cr. It was then transferred into a separating funnel and the 500 mL of n-hexane was added into suspension. It was put in the separating funnel for 6 hours after vigorous shaking. The two layers obtained after a while was separated in which the upper layer obtained was n-hexane. The *n*-hexane fraction was obtained by repeating this process several times until the upper layer obtained was completely transparent. Similarly fractionation was processed for chloroform, ethyl acetate and at the end aqueous fraction was left. The Rh.Hex, Rh.Chf, Rh.EtAc and Rh.Aq obtained were 30, 22, 53and 140 grams [14].

Extraction of crude saponins

For the extraction of crude saponins from *Rumex hastatus*, 20 grams of plant sample was added to a conical flask and 100 mL of 20% ethanol was added to it. The sample was heated at 55°C in the water bath for 4 hours with continuous shaking. With the completion of 4 hours the sample was filtered and

the residue obtained was re-extracted with 200 mL of 20% ethanol.

The sample obtained was heated until the volume was reduced to 40 mL. The concentrated volume obtained was transferred into the separating funnel and after addition of 20 mL of diethyl ether, the whole volume was vigorously shaken in the separating funnel, to obtain two layers the separating funnel was put calm for a while. The lower aqueous layer was collected while the upper diethyl ether layer was discarded. Then 60 mL n-butanol was added into the aqueous portion and the combined *n*-butanol extract was washed with 10 mL of 5% sodium chloride solution. The final solution obtained was kept in a hot water bath until the solvent was fully evaporated and the saponins obtained were dried using oven at low temperature and the dried saponins obtained were 1.3 grams[16].

Anti-Pharaoh ant activity

This activity was performed by the direct contact toxicity method, following the procedure of Ahn et al (1995) [17]. Solutions of different concentrations of Rh.Cr, resultant fractions and Rh.Sp were prepared in distilled water. The concentrations prepared of each sample were 2, 4, 6 and 8 mg/ml. Each concentration was poured into petri dishes containing sterilized filter paper and placed overnight for evaporation of solvent. After evaporation 30 pharaoh ants were added into each petri dish. The petri dishes containing filter paper plus distilled water served as a control. The petri dishes were placed at room temperature for 24 hours and by the completion of this duration, the total number of dead and alive ants was counted in each petri dish. Each sample was treated in triplicate. The percent mortality of each sample was calculated and the data was arranged in the table as mean ± S.D.

Anti termite activity

The anti-termite activity of Rh.Cr, Rh.Sp and resultant fractions (Rh.Hex, Rh.Chf, Rh.EtAc, Rh.Aq) of *Rumex hastatus* was conducted following the procedure of Salihah et al. [18]. In this procedure, plant extracts were assayed against *Heterotermes indicola*. Sterilized filter paper equal to the size of the petri dishes were cut. Samples were prepared by dissolving 2 mg/ mL of each extract in respective solvents. To evaporate the solvent, the filter papers were kept overnight. Each filter paper was kept in respective labeled petri dishes. Then 25 termites were added to each petri plate and kept for 24 hours at room temperature. By the completion of 24 hours the number of dead and alive termites was counted. Similarly, the results were recorded accordingly on the second and third day. This experiment was performed in triplicate and the average termites killed each day were recorded.

Statistical data analysis

All the tests were performed in triplicate and values were expressed as means ± S.D. LC50 was calculated using Microsoft Excel 2007. Significance between percent lethality and test samples were analyzed using GraphPad Prism software. Group comparison was performed by Student's *t*-test in which the $P < 0.05$ were considered significant.

Results

Anti-Pharaoh ant effect

The anti-Pharaoh ant activity of Rh.Cr, Rh.Sp and resultant fractions demonstrated excellent potential of *R. hastatus*. The Rh.Cr exhibited 56.66 ± 2.88, 81.66 ± 5.77 and 93.33 ± 2.88% lethality at the concentrations of 4, 6 and mg/mL respectively after 24 hours with LC_{50} 3.7 mg/mL. All concentrations of Rh.Hex, Rh.Chf, Rh.EtAc and Rh.Aq showed a dose dependent activity. The order of percent lethality of fractions was Rh.Chf> Rh.EtAc> Rh.Aq> Rh.Hex showing 100.00 ± 0.00 > 93.33 ± 2.88 > 83.33 ± 2.88 > 83.33 ± 2.88% lethality respectively at highest concentration (8 mg/mL). Rh.Sp showed sterling activity killing almost all the Pharaoh ants at all the concentrations. The order of activity of samples were Rh.Sp> Rh.Chf> Rh.EtAc> Rh.Cr> Rh.Aq> Rh.Hex as shown in the table.1. The LC_{50} calcualted was lowest (LC_{50} < 0.01 mg/mL) for Rh.Sp, second lowest for Rh.Chf (LC_{50}< 0.1 mg/mL) and highest (LC_{50} = 4.0 mg/mL) for Rh.Hex.

Anti-termite effect

The anti-termite activity was performed for Rh.Sp, Rh.Hex, Rh.Chf, Rh.EtAc, Rh.Aq and Rh.Cr of *Rumex hastatus* against the *H. indicola*. Each experiment was performed in triplicate and the results obtained were arranged in the table 2. The Rh.Sp and Rh.Chf excelled among all samples. The Rh.Sp, Rh.Cr, Rh.Hex, Rh.Chf, Rh.EtAc and Rh.Aq exhibited 100.0 ± 0.00, 49.32 ± 2.28, 56.00 ± 4.00, 93.32 ± 2.28, 80.00 ± 0.00 and 46.64 ± 6.12% lethality respectively on the first day.Similarly on the second day, Rh.Sp, Rh.Cr, Rh.Hex, Rh.Chf, Rh.EtAc and Rh.Aq exhibited 100.0 ± 0.00, 73.32 ± 2.28, 100.0 ± 0.00, 97.32 ± 2.28, 70.64 ± 2.28% lethality. Almost all the termites were killed by all the samples on the third day. The overall anti-termite effect of Rh.Sp was awesome and among the fractions Rh.Chf and Rh.EtAc showed

magnificent activity while the remaining fractions showed moderate anti-termite activity.

Discussion

As inferred from the observations in both activities, the *R. hastatus* revealed strong anti-pharaoh ant and anti-termite effects. As previously reported, the saponins possess strong insecticidal potential [19], our results also goes parallel, showing remarkable activity against pharaoh ants and termites. Similarly, a strong correlation exists among the results of various fractions for both the insects. The correlation value (R = 0.943), indicates that against both insects the lethality ratio of various samples of *R. hastatus* are equivalent upto considerable extent.

As the northern areas of Pakistan are famous for natural beauty and the natural beauty is due to the forests consisting of different types of valuable trees which are the best source of furniture, medicines, fuels, timbers, fodder etc [20]. Numerous termites are found in the soil of these forests and these termites play an important role in the decomposition of cellulose present in the soil as waste materials but on the other hand these termites cause a huge destruction by eating the bark of valuable trees and thus terminating the life of trees by destroying the base of trees and ultimately leads to huge economic loss [21]. Similarly in the homes these termites destroy valuable goods and the pharaoh ants destroy and contaminate food materials. The saponins may kill insects by impairing the digestion of the insects by slowing down the passage of food through the gut [22]. Another possible mode of action of saponins is that it decrease the absorption of sterols, as the insects cannot biosynthesize the sterols but they need it from the outside so they need sterols to synthesize ecdysteriods and also for the synthesis of hydroxyecdysone responsible for moulting [23]. Saponins have been identified and isolated from some of the species of the family leguminoseae [24], solanaceae [25], theaceae [26], alliaceae [27], chenopodiaceae [28]. various pharmacological activity have been performed on various plants demonstrating the importance of flora for living beings [29]. All the reported evidences and the scientific verification clarify that the saponins may be the basic components of plant sample responsible for insecticidal activity.

Based on the literature survey and the results of this study, it may be inferred that the Rh.Sp isolated from *Rumex hastatus* can be a suitable source of natural insecticides, the various separation and purification techniques should be applied to Rh.Sp, which may lead to isolation of bioactive compounds having insecticidal potential. The Rh.Chf may also be a best source of insecticidal drugs, so it should be subjected further to various chromatographic techniques, to isolate the target compound responsible for the insecticidal activity.

Acknowledgments

The author is grateful to the taxonomist for the identification of plant species and the head of Department of Pharmacy for accomplishment of this activity

References

1. Aronson, E., The social animal. 2003: Worth Pub.
2. Eaton, R.A., Hale, M.D., Wood: decay, pests and protection. 1993: Chapman and Hall Ltd.
3. McKinney, D., Solving nuisance ant problems without pesticides. J Pest Ref 2002;22:3
4. Holman, H.J., A survey of insecticide materials of vegetable origin. A Survey of Insecticide Materials of Vegetable Origin. 1940.
5. Hill DS, Pests of stored products and their control. 1990: Belhaven Press.
6. Battu, R., Singh, B., Kang, B., Joia, B., Risk assessment through dietary intake of total diet contaminated with pesticide residues in Punjab, India, 1999–2002. Ecotoxicol Environ Saf 2005;62:132-139.
7. Visetson, S., Purivirojkul, W., Kannasutra, P., Insecticide resistance mechanisms in various strains of the rust red flour beetle (*Tribolium castaneum* Herbst). Warasan Witthayasat Kaset. 2002;33.
8. Thummasarangkoon, W., Efficiency of lemon grass (*Cymbopogon winterianus* Jewitti) extracts on mortality and the levels of detoxification enzymes in larva of *Culex pipien* quinquefasciatus. 2000, MS thesis, Kasetsart University, BKK Thailand.
9. Saekin, P., Effect of Leaf Extracts From *Eupatorium odoratum* L. on Detoxification Enzymes Levels in Golden Apple Snail (*Pomacea canaliculata* L.). 2000, MS thesis, Kasetsart University.
10. Ruamthum, W., Effect of Nutgrass (*Cyperus rotundus* L.) Corn Extracts on Esterase and Glutathione-S-Transferase Level in Digestive System of the Golden Apple Snails (*Pomacea canaliculata* Lamarck). 2002, MS thesis, Kasetsart University.
11. Rao, K., Sunitha, C., Banji, D., Sandhya, S., Mahesh, V., A study on the nutraceuticals from the genus Rumex. Hygeia JD Med. 2011;3:76-88.
12. Francis, G., Kerem, Z., Makkar, H.P., Becker, K., The biological action of saponins in animal systems: a review. Br J Nutr 2002;88:587-605.
13. Shah, S.M.M., Sadiq, A., Shah, S.M.H., Khan, S., Extraction of saponins and toxicological profile of *Teucrium stocksianum* boiss extracts collected from District Swat, Pakistan. Biol Res 2014;47:65.
14. Zeb, A., Sadiq, A., Ullah, F., Ahmad, S., Ayaz, M., Phytochemical and toxicological investigations of crude methanolic extracts, subsequent fractions and crude saponins of *Isodon rugosus*. Biol Res 2014;47:57.
15. Ayaz, M., Junaid, M., Subhan, F., et al. Heavy metals analysis, phytochemical, phytotoxic and anthelmintic

investigations of crude methanolic extract, subsequent fractions and crude saponins from *Polygonum hydropiper* L. BMC Complement Altern Med 2014;14: 465.
16. Ayaz, M., Junaid, M., Ahmed, J., et al., Phenolic contents, antioxidant and anticholinesterase potentials of crude extract, subsequent fractions and crude saponins from *Polygonum hydropiper* L. BMC Complement Altern Med 2014;14:145.
17. Shah, S.M.M., Sadiq, A., Biological activities of crude saponins, methanolic extract and sub fractions of *Teucrium stocksianum* Bioss collected from north west of Pakistan. 2014.
18. Salihah, Z., Khatoon, R., Khan, A., Alamzeb, S.A., A termite trap, NIFATERMAP, for capturing large number of field population of *Heterotermes indicola*. in Proc. Pakistan Cong Zool 1993.
19. Tava A and Odoardi M, Saponins from Medicago spp.: chemical characterization and biological activity against insects, in *Saponins used in food and agriculture*. Springer 1996;97-109.
20. Myers, N., Tropical forests: Much more than stocks of wood. JTrop Ecol 1988;4:209-221.
21. Yudin, L., Termites of Mariana Islands and Philippines, their damage and control. Sociobiology. 2002; 40: 71-74.
22. Adel, M.M., Sehnal, F., Jurzysta, M., Effects of alfalfa saponins on the moth Spodoptera littoralis. J Chem Ecol 2000;26:1065-1078.
23. Bellés, X., Martín, D., Piulachs, M.D., The mevalonate pathway and the synthesis of juvenile hormone in insects. Annu Rev Entomol 2005;50:181-199.
24. Kapusta, I., Janda, B., Stochmal, A., Oleszek, W., Determination of saponins in aerial parts of barrel medic (*Medicago truncatula*) by liquid chromatography-electrospray ionization/mass spectrometry. J Agric Food Chem 2005;53:7654-7660.
25. Kohara, A., Nakajima, C., Yoshida, S., Muranaka, T., Characterization and engineering of glycosyltransferases responsible for steroid saponin biosynthesis in Solanaceous plants. Phytochemistry 2007;68:478-486.
26. Kohata, K., Yamauchi, Y., Ujihara, T., Hprie, H., Growth inhibitory activity of tea-seed saponins and glyphosate to weed seedlings. Jpn Agr Res Q 2004;38:267-270.
27. Matsuura, H., Saponins in garlic as modifiers of the risk of cardiovascular disease. J Nut 2001;131:1000S-1005S.
28. Massiot, G., Dijoux, M-G., Lavaud, C., et al., Seco-glycosides of oleanolic acid from *Beta vulgaris*. Phytochemistry 1994;37:1667-1670.
29. Zeb, A., Sadiq, A., Ullah, F., Ahmad, S., Ayaz, M., Investigations of anticholinestrase and antioxidant potentials of methanolic extract, subsequent fractions, crude saponins and flavonoids isolated from *Isodon rugosus*. Biol Res 2014;47:1-10.

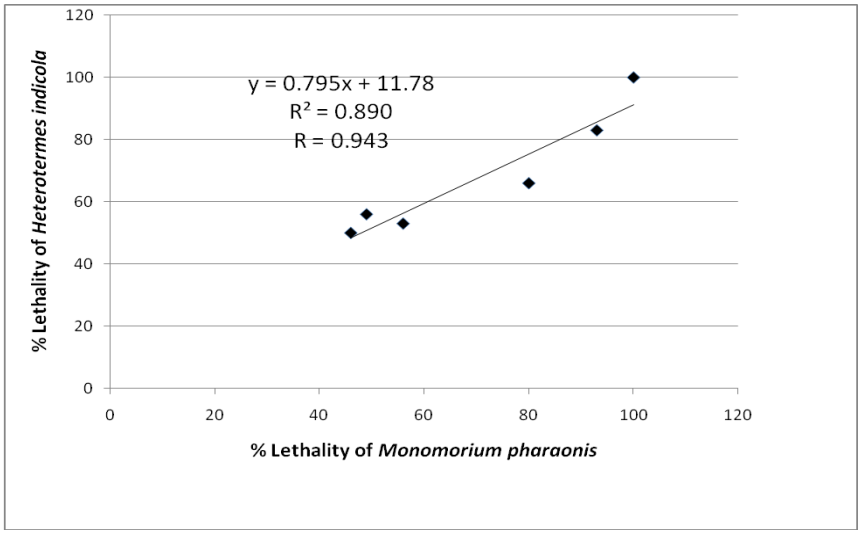

Figure 1. Linear correlation of % Lethality of *Heterotermes indicola* Vs *Monomorium pharaonis*

Table 1. Anti pharaoh ant effect of various extracts of *Rumex hastatus*

Samples	Dose (mg/ml)	Total treated	No. Repeated	Percent lethality (mean±S.D)	LC_{50} (mg/ml)
DW		20	3	0.00	0.00
Rh.Cr	4	20	3	56.66 ± 2.88***	3.2
	6	20		81.66 ± 5.77***	
	8	20		93.33 ± 2.88***	
Rh.Hex	4	20	3	50.00 ± 5.00***	4.0
	6	20		68.33 ± 5.77***	
	8	20		83.33 ± 2.88***	
Rh.Chf	4	20	3	83.33 ± 2.88***	<0.1
	6	20		93.33 ± 2.88***	
	8	20		100.00±0.00	
Rh.EtAc	4	20	3	66.66 ± 7.63***	1.6
	6	20		76.66 ± 5.77***	
	8	20		93.33 ± 2.88***	
Rh.Aq	4	20	3	53.33 ± 2.88***	3.4
	6	20		71.66 ± 7.63***	
	8	20		83.33 ± 2.88***	
Rh.Sp	4	20	3	100.0 ± 0.00	<0.01
	6	20		100.0 ± 0.00	
	8	20		100.0 ± 0.00	

Values are expressed as mean ± SD; n=3; *: P < 0.05, **: P < 0.01, ***: P < 0.001.
Key: DW = Distilled water; Rh.Cr = Crude methanolic extract; Rh.Hex = *n*-hexane fraction; Rh.Chf = Chloroform fraction; Rh.EtAc = Ethyl acetate fraction; Rh.Aq = Aqueous fraction; Rh.Sp = Crude Saponins.

Table 2. Anti-termite effect of various extracts of *R. hastatus*

Samples	Total termites treated	Days	Percent lethality (mean±S.D)
Rh.Cr	25	1	49.32 ± 2.28***
		2	73.32 ± 2.28***
		3	100.0 ± 0.00
Rh.Hex	25	1	56.00 ± 4.00***
		2	65.32 ± 4.60***
		3	100.0 ± 0.00
Rh.Chf	25	1	93.32 ± 2.28***
		2	100.0 ± 0.00
		3	100.0 ± 0.00
Rh.EtAc	25	1	80.00 ± 0.00***
		2	97.32 ± 2.28
		3	100.0 ± 0.00
Rh.Aq	25	1	46.64 ± 6.12***
		2	70.64 ± 2.28***
		3	100.0 ± 0.00
Rh.Sp	25	1	100.0 ± 0.00
		2	100.0 ± 0.00
		3	100.0 ± 0.00

Values are expressed as mean ± SD; n=3; *: P < 0.05, **: P < 0.01, ***: P < 0.001.
Key: Rh.Cr = Crude methanolic extract; Rh.Hex = *n*-hexane fraction; Rh.Chf = Chloroform fraction; Rh.EtAc = Ethyl acetate fraction; Rh.Aq = Aqueous fraction; Rh.Sp = Crude Saponins.