J. Basic. Appl. Sci. Res., 4(1)179-184, 2014

ISSN 2090-4304
Journal of Basic and Applied Scientific Research
www.textroad.com

Molluscicidal Activity of Aqueous Leave Extract of *Solenostemma argel* (Del Hayne) on *Biomphalaria pfeifferi* Snails

Ahmed, E.A[1]; Babiker, O.F[2] and Abdalla, R.M[3]

[1] University of science and Technology, Department of medical sciences, Biology unit
[2]ElnileinUniversity, Faculty of science
[3] Nile College, Biology Department

Received: October 20 2013
Accepted: November 30 2013

ABSTRACT

Schistosomiasis is one of the most common parasitic diseases that causes mortality in tropical countries, and has been targeted for increased control strategies. These are based on health education, selective population-chemotherapy, improvement of water supplies and sanitation, and also the snail control. Use of molluscicides to eradicate the snail vector is considered the best choice to eliminate schistosomiasis .It includes either synthetic molluscicides or those of natural origin.Molluscicides of plant origin have gained greater importance since it is believed that natural products are ecologically and culturally sound than synthetic ones. In addition, the use of local plants as molluscicides is beneficial in reducing burden of purchasing expensive synthetic molluscicides used in snail control. Molluscicides greatly affect the metabolic activities of the snail intermediate hosts.

The main objectives of this study was to evaluate the molluscicidal activity of aqueous leaf extract of *Solenostemm a argel* (Del Hayne) plant against adults of *Biomphalaria pfeifferi* snails under laboratory conditions.

The results of mortality were statistically analyzed using probit analysis to estimate the LD_{50} and LD_{95}. The values of the lethal concentration LD_{50} and LD_{95} were found to be 0.103 and 0.187 ppm respectively.

Phytochemical screening of water and ethanolic extracts of *S .argel* Del Hayne leaves were performed, and it confirmed the presence of saponine, tannins, flavonoids, alkaloids, terpenoides and cardiac glycocides.

KEY WORDS: Schistosomiasis, Molluscicidal, Asclepiadaceae, snails, Sudan

I: INTRODUCTION

In Sudan, Shistosomiasis is considered to be one of the major health problems, and it spreads parallel with the expansion in agricultural irrigation dams, pump and schemes which create suitable habitat for the intermediate hosts “snails [1]. Poor communities are much threatened by this disease, because health resources are most limited and people lack access to safe drinking water and adequate sanitation [2].The causative agents of the disease are the blood trematodes of the genus Schistosoma. These parasites undergo part of their development in fresh water snails, which serve as intermediate hosts [3]. Some 350 snail species are estimated to be of possible medical or veterinary importance. Most intermediate hosts of human *Schistosoma* parasites belong to three genera, *Biomphalaria*, *Bulinus* and *Oncomelania*.

Control could be taken by breaking the life cycle and hence, preventing further development of the disease. To do this, alternative and inexpensive chemical compounds with molluscicidal properties could be sought to accomplish physical destruction of snails from water bodies [4].Plant molluscicide could be appropriate for snail control measures against schistosomias is in endemic areas, as they may be highly effective, and less expensive than synthetic molluscicides [5].

Sudan has a wide range of ecological habitats and vegetational zone. Hargel (*Solenostemma argel*) is a desert plant of traditional medical uses in the Sudan, which is regarded as the richest source of this plant.

Various studies had been performed on the insecticidal activity of Hargal plant (Solenostemma argel /Del Hyne) on several insect species, e.g.: [6, 7, 8], but less studies have been done to test its mollucicidal activities. It grows wild in the area extending from Dongola to Barber, particularly around Abu Hamad, where it is grown under irrigation [9].

In this study, Hargal plant was used to assess its mollucsicidal activity on *Biomphalaria pfeifferi* snails.

MATERIAL AND METHODS

1. Snails:

This study was conducted on adults of Biomphalaria *pfeifferi* snails, (5-7mm) in diameter.

1.1 Collection and Sampling:

Snails were collected from water bodies in different locations around Khartoum State, and then taken to the Laboratory, for breeding and maintenance.

***Corresponding Author:** Dr. Elham Abdalla Ahmed, University of Science & Technology, Department of Medical Engineering, Biology Unit/e.mail:aaya2001@hotmail.com

1.2 Breeding and Maintenance:

Collected snails were screened for the presence of natural trematodes infection. All uninfected *Biomphalaria* snails were maintained in separate plastic aquaria containing about 10 liters of dechlorinated - tap water at room temperature, (25° - 30°C). The aquaria were subjected to fluorescent light for a period of about 12 hours daily. The snails were fed fresh lettuce or chard leaves in reasonable quantities every second day, and water was changed once a week, according to need. Dead snails were removed when observed.

2 Plant used: *Solenostemma argel* Del (Hayne):

It belongs to family Asclepiadaceae, and has characters common for most of its genera, (milky juice, opposite leaves without stipules, corolla double with a small (corona) inside of various shapes, seed dispersal by hairs.

2.1 Collection and Preparation of Plant Material:

Dry leaves of *S.argel* were obtained from local markets and cleaned manually to remove dust and any unwanted materials. They were then ground using an electric blender (Moulinex).The powder obtained was stored in glass jars covered with plastic covers, and left at room conditions.

2.2 Preparation of Aqueous Plant Extract:

A stock solution of 5% (5 grams of powder in 100 milliliters of distilled water) was prepared and kept in Stoppard bottles. Mixing was carried out in a conical flask on a magnetic stirrer for 6 hours with the flask shaken every 30 minutes. After 24 hours, the solution was filtered through cotton wool, and used within 3 days.

2.3 Exploratory Trials:

These were conducted in accordance with the guidelines of the [10]. Widely logarithmic spaced doses (concentrations) were prepared as experimental solutions**,** by adding different appropriate volumes from the stock solution to 250 ml of de-chlorinated tap water in separate small Aluminum dishes. Then 10 snails were immersed in each dish. The exposure time was 24 hours, because no further changes were detected after that time duration, and the toxic effects of the active plant extract became evident in the tested snails. It was followed by a recovery period of 24 hours in dechlorinated tap water. Once the extent of the toxicity range was determined, several convenient concentrations of the stock solution were prepared (dilution with dechlorinated tap water) to give mortalities between 0 to 100%.

2.4 Detection of snail death:

A snail was considered dead when floating with one of its flat sides uppermost onto the surface of the water with its shell becoming translucent. Some snails settled motionless at the bottom of the dish,. Confirmation of the snail death was ensured when it settled motionless into the bottom of the bowl with no response to photo stimulation and mechanostimulation [11].

2.5 Potency tests of aqueous plant extract:-

For each chosen concentration of the aqueous extract, 20 adult snails (5-7mm) were placed in each dish, containing 250 ml of dechlorinated tap water [10] .The exposure and recovery periods are the same as mentioned above. Parallel control experiments were carried out using dechlorinated tap water. No food was provided during the exposure test. The behavioral responses of the tested individuals were monitored every 10 minutes for the first hour then every hour for the next 6 hours. Thereafter the responses recorded occasionally until the elapse of the 24 hours. The total numbers of the dead snails were recorded.

For each dilution of the extract, the above experimental steps were repeated twice.

3. Phytochemical Analysis of *S.argel* Extracts:

3.1 Preparation of the plant extracts:

50 g of the powdered leaves of hargel plant was extracted in a Soxhlet sequentially in 300 ml of ethanol; water. The process was run for 48 hrs. After which the sample was concentrated using rotary evaporator and freezed, dried to powdered form. The dried extract was weighed and kept in a labeled sterile specimen bottle [12].

3.2 Preliminary phytochemical tests:

Phytochemical screening was performed using standard procedures [12].

3.2.1 Test for Anthraquinone:

0.5 g of the extract was boiled with 10 ml of sulphuric acid, filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for color changes.

3.2.2 Test for Terpenoids: (Salkowski test)
0.5 g of the extract was added to 2 ml of chloroform, 3 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

3.2.3 Test for Tannins: (Ferric chloride test)
0.5 g of the extract was boiled in 10 ml of water in test tube , and then filtered. A few drops of 0.1 % ferric chloride was added and observed for brownish green or a blue – black coloration

3.2.4 Test for Saponins: (Froth test)
5.0 ml of distilled water was added to 0.5 g of the extract in, a test tube. The solution was shaken vigorously and observed for a stable persist froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, observed for the formation of the emulsion.

3.2.5 Test for Alkaloids:
0.5 g of the extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloid base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and draggendroffs reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendroff's reagent) was regarded as positive for the presence of alkaloid.

3.2.6 Test for Cardiac glycosides: (Keller-Killiani test)
0.5 g of the extract was diluted to 5 ml with water. Then 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

4. Statistical analysis:
This was done using SPSS statistical package version16. The data also were subjected to analysis by probit regression.

RESULTS

1. **The effect of *S.argel* aqueous leaf extract on adult snails:** Table (I) shows the upper and lower limits of the 24-hours LD_{50} and LD_{95} aqueous extracts of *S.argel* on the snails. The number of dead snails in each applied dose of the extract is shown in fig (1).
Lethal doses (LD_{50} and LD_{95}), were obtained usingprobit analysis [13]. The plot probit of kill against log of doses provides a simple graphic representation of the dose-to- response ratio. The probit mortality shows a linear relationship with the log concentration of aqueous extract of *S.argel* leaves, (fig.2).

Table (I): **The 24-hours LD_{50}&LD_{95} of the aqueous extract of (*S.argelDel Hayne*) on adults of *B.pfeifferi***

Probability	*Confidence limit for Dose*		
	Estimate	*Lower bound*	*Upper bound*
LD_{50}	0.103	0.088	0.119
LD_{95}	0.187	0.153	0.275

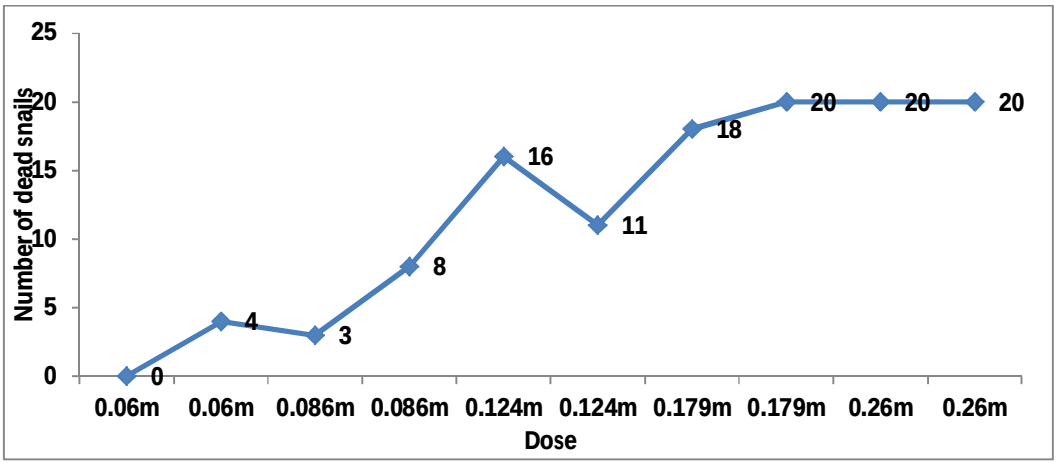

Fig.1: **Number of dead snails per dose of aqueous extract of *S.argel* leaves**

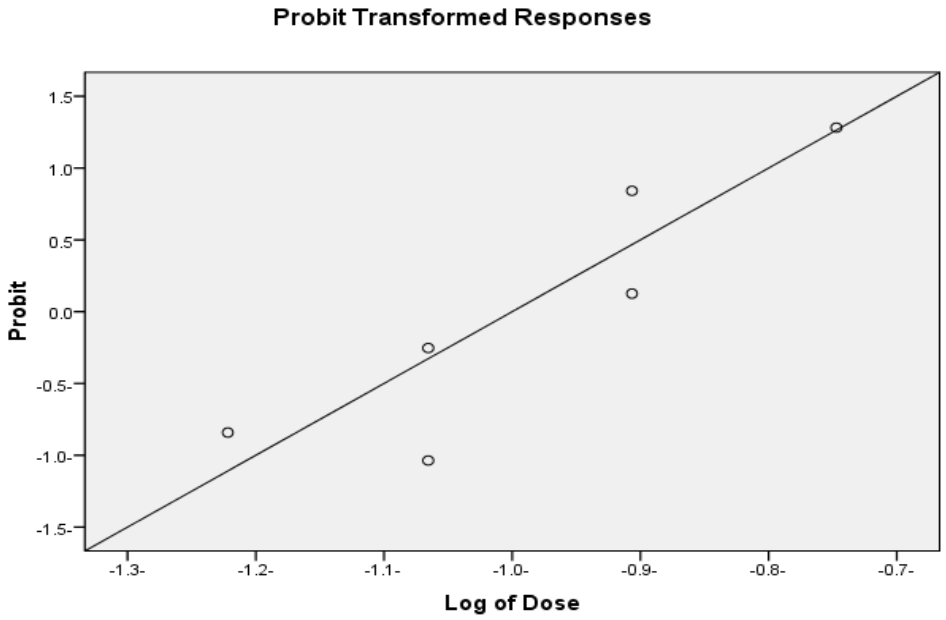

Fig.2: **Log dose /probit regression line of *S.argel* Del Hayneaqueous extract of leaves on adult of *B. pfeifferi***

PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000logarithm.)

= 6.26 + 6.33x

Intercept = 6.26

Coefficient of dose = 6.33. the slope of the model

This means that an increase in Dose score increases the predicted probability of death

2. Phytochemical screening of plant materials:

The phytochemical screening of the plant studied showed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids and cardiac glycosides, in alcohol and water extracts, (see, table2). The presence of Anthraqinones was not detected in both extracts.

Table 2**: Phytochemical constituents o f(*S.argel* Del Hayne) leaves**

Secondary metabolite	Alcohol extract	Water extract
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Anthraquinones	-	-
Trepenoids	+	+
Cardiac glycocides	+	+

3-Observations regarding responses of snails:

Snails in the untreated dishes and control withdrew into their shell but became active after a while, moving around the container. Immediately after the application of the extract, or after some seconds, depending on the extracts `concentrations, the snails withdrew into their shell, but they start crawling out of the water, and cluster together most staying at the water-air interface with their shell partially immersed in the water. After 24hrs, the toxic effects of the active plant extract became evident in the test snails. Partially dead snails retracted partially into their shells (withdrawal response) or no retraction in the dead snails. Development of hemorrhagic “blisters” on the ventral surface of some of the snail was also noted.

DISCUSSION

Transmission reduction via intermediate snail host is still considered as the most important means of schistosomiasis control. The use of local plants as molluscicides is beneficial in reducing burden of purchasing expensive synthetic molluscicides used in snail control. A large number of plant families, which possess natural molluscicidal activity, have been identified [14, 15, 16].However, according to [17], Asclepiadaceae was placed among the important flora families showing several bioactive plant species in Sudan. In the present investigation, (*S.argel* Del Hayne) which is a member of

this family was used. The obtained results revealed that adult *B.pfeifferi* snails were sensitive to water extracts from leaves of Hargal plant (*S.argel* Del Hayne). Hence, water extracts of leaves of this plant, has molluscicidal properties and can be used as a molluscicide in the control of schistosome-snail intermediate host.

The LD_{50} and LD_{95} values were used to quantify the levels of the plant toxicity. The LD_{50} was 0.103 ppm and the LD_{95} was 0.187 ppm. Molluscicidal activities of different parts of other plant species against *B.pfeifferi* showed variation in toxicity. The results of this study showed the best LD_{50}, when compared to that of [18] who found that the LD50 of crude extracts from *Jatropha. Elliptica* was 24.8 ppm against *B. glabrata* adults. Also[19], found a higher value of (LD_{50}) of crude aqueous extracts of ground seeds of *J.curcas* against, *B.pfeifferi*, which was 50 ppm. Being a member of the same family Asclepiadaecae as S.argel, *Calotropisprocera* showed the best LC_{50} and LC_{95} than *Nicotianatabacum* against adult *Bulinustruncatus* snails. The values for *C.procera* were 619ppm and 1100ppmrespectively, while those of *Nicotianatabacum* were 804 and 1386ppm respectively [20].

The insecticidal potentiality of *S.argel* was investigated by many workers.[8] used the aqueousextracts of *S.argel* as a larvicide of two mosquitoe species, and it showed the best LD_{50} when compared with *Calotropisprocera*, which is a member of the same family.[21] showed that extract of the fruit pericarp of *S.argel* was most effective when tested for larvicidal activity against the third instar larvae of the mosquito *Culexquinquefasciatus* Say., where its $LC_{50}$was the best when compared with that of the crude aqueous extracts of flower, root and stem .

The behavior of each snail in the untreated water in the dishes and control i.e. (withdrawal into their shell and subsequent activity after that), agrees with those reported by[21].The withdrawal of the snails again into their shells, when the extracts were introduced and their crawling to the water- air interface, was similarly reported by [22].

Phytochemical tests showed the presence of saponins, tannins, flavonoids, terpenoids, alkaloid and cardiac glycosides in ethanolic and water extracts of *S.argel*. They may have contributed to the molluscicidal activity of this plant on the snails [8].Such results agree with many investigators.[23] showed that flavonoids, alkaloids and glycoside were found in varying amounts in ethanolic and aquatic S.argel extracts. [24] also detected the presence of saponins, flavonoids, tannins, sterols, glycocidesterpenes, as the main constituents in *S.argel*.[25], found the presence of glycocides together with other active substances in the *Solenostemma* argel by phytochemical study.[26] showed the presence of saponins, alkaloids, glycosides as the major components of *S. argel* aqueous extracts.

It is well known that the chemical poisons from plants such as Hargel (S. *argel)* and Usher *C. procera,* which belong to the same family (Asclepiadaceae), are mostly alkaloids. Alkaloids are plant products, which are nitrogenous in nature. They are heterocyclic compounds having strong effects on the nervous system of animals and which may result in death, [27, 28].

An important advantage of using extracts from the Hargel plant in the control of Schistosoma's snail intermediate host, is that it is a natural product which is locally abundant and can be prepared at low cost. This economic factor is of great importance in many regions endemic for schistosomiasis infection.

CONCLUSION

From the present study, it can be concluded that the aqueous extract of *S.argel* Del Hayne leaves has molluscicidal properties and can be used as a molluscicide in the control of schistosomiasis.

This make the experiment another addition to experiments carried out to investigate the use of plant products in controlling the Schistosoma snail intermediate host so as to prevent schistosomiasis infection.

Acknowledgements:

The technical assistance and guidance of all the staff of the Reference laboratory for the control of Bilharziasis is very much acknowledged..

REFERENCES

1-World Health Organization, 2003. Action against worms: report series- issue 1.

2 - King, C.H. ,2010. Parasites and poverty: the case of schistosomiasis. *Acta Trop*, 113: 95–104.

3- Yirenya, T. ,I., A., Aboagye, G.,Abdul Rashid, M., Futagbi, M. Dade , 2011. Prevalence of Snail Vector of Schistosomiasis in the Kpong Head Pond, Ghana. WAJ E,18:40-45

4- Fransworth, N.R., I.O., Henerson, D.D.,Soejarto, 1987. Plants with Potential Molluscicidal Activity. In KE Mott, Plant Molluscicides .ChichesterAnd Elsewhere. John Wiley And Sons Limited, New York. 131-204.

5- Schall, V.T., M.C. ,Vasconcellos, R.S., Rocha, C.P., Souza, N.M., Mendes, 2001. The control of the schistosome-transmitting snail Biomphalariaglabrata by the plant molluscicide*Euphorbia splendens*Var. hislopii (synmilli Des.Moul): Alongitudinal field study in an endemic area in Brazil, ActaTropica 79:165–170.

6- Abdel-Rahman H.R&R.N, .Al-Mozini, 2007. Antifeedant and toxic activity of some plant extracts against larvae of cotton leafworm Spodoptera littoralis (Lepidoptera: Noctuidae). Pak.JBiol Sci., 10(24):4467-72

7- Sidahmed, O.A.A., A.K., Taha, H.G.Mardi, T.I.M., Idris, 2009. The efficiency of spraying date palm trees with argel (*Solanostema argel* Hayne) for the control of the white scale insect (*Parlatoriablanchardii* Targ.) (Homoptera, Diaspididae).JUST. 10 (1): 142- 149.

8- Hag El Tayeb, F. M., A.K., Taha, H.G.,Mardi, O.A.A., Sidahmed,2009. Water extracts of Hargel plant (*Solenostemmaargel* Del Hyne) and Usher (*Calotropisprocera*Ail) leaves as naturalinsecticides against mosquito larvae. JUST, 10 (3): 59-67.

9- EL-Kamali, H.H., R.O., EL-Nour, S.A.,Khalid ,2010. Molluscicidal Activity of the Essential Oils of *Cymbopogonnervatus* Leaves and *Boswelliapapyrifera* Resins -Curr. Res. J. Biol. Sci 2(2): 139-142.

10-World Health Organization, 1965. Molluscicide screening and evaluation. Informal meeting of investigators on molluscicide screening and evaluation held during 17-21 November 1964. Geneva.

11- Otarigho, B. &O.A., Morenikeji, 2013. Molluscicidal Effects of Aqueous and Ethanolic Extracts of Lemongrass (*Cymbopogon Citratus*) Leaf against the Different Developmental Stages of *BiomphalariaPfeifferi*. NY Sci J,5(8):70-77.

12-Harborne, J.B.,1998. Phytochemical methods: A guide to modern technique of plant analysis, Champman and Hall, London.

13- Finney, D. J. ,1971. ProbitAnalysis. 3. ed. Cambrige: Cambridge University Press, 333pp.

14- Abdel-Gawad , M. M. ,H.A., El-Nahas, and E. S. Abdel-Hameed, 2004. Laboratory evaluation of the molluscicidal ,miracidial and cercarial properties of two Eguptian plants. Bull. Pharm. Sci., Assiut University. 27(2),:331-339.

15- Elnahas, H.A, F.A.Eldeeb , 2007. Molluscicida potency of Pittosporum Tobirta Varigatum and Hedera Canariensis plants against juvenile and adult Biomphalaria Alexandrina snails. Egypt J. AquatBiol&Fish., 11 (l):151-17

16- Hasheesh, W.S., R.T., Mohamed, Abd El-Monem,S., 2011. Biological and physiological parameters of *Bulinustruncatus* snails exposed to methanol extract of the plant *Sesbaniasesban* plant., ABC, 1: 65-73.

17- Satti A.A.,H.A., Hashim, O.E., Nasr, 2010. Biologically active chemicals with pesticidal properties in some Sudanese plants J. Int. Environ. Appl. Sci., 5 (5) : 767–780

18- Santos, A. F., and A.E. G. Sant'Ana, 2000.The molluscicidal activity of plants used in Brazilian folk medicine. *Phytomedicine* 6:431–438.

19- Amin, M. A.,A.A., Daffalla, and O.A. el-Moneim,1972. Preliminary report on the molluscicidalproperitieis of *Habat El Mulluk, Jatropha*sp. Trans. R. Soc. Trop. Med. Hyg.66:805–806.

20- El-Kamali, H.H., 2001. Larvicidal activity of crude aqueous extracts of *Solenostemmaargel*against mosquito larvae. J. Herb. Spices Med. Plants, 8(4): 283-286.

21- Gehad T. E, A.Z.,RawiaandT.E.,Eman,2009: Molluscicidal Activity of Some *Solanum* Species Extracts against the Snail *Biomphalariaalexandrina*, J Parasitol Res.,2009:5pp.

22- Adetunji V.O, O.T.Salawu., 2010. Efficacy of ethanolic leaf extracts of Carica papaya and Terminaliacatappa as molluscicides against the snail intermediate hosts of schistosomiasis. J. Med.Plant. Res ;4(22):2348-2352.

23-Abou-Hashem, A.A.M, 2013 :Rodenticidal effect of Argel (*GomphocarpussinaicusBoiss*) leaves on the Norway rat(Albino), *Rattusnorvegicus*, Berkenhout under laboratory conditions. J. Appl. Sci. Res , 9(3): 1690-1695

24- Kamel, M.S., H.A ,Ohtani, M. H., Hasanain, M., R. Kasai and K. Yamasaki, 2000. Monoterpene and pregnane glycosides from Solenostemmaargel. Phytochemistry, 53: 937-940

25-Al-Doghairi, M., A. EL-Nadi, E. Elhag and H. Al-Ayedh, 2004. Effect of Solenostemmaargel on oviposition, egg hatchability and viability of Culexpipiens L. larvae. phytother Res.,18: 335-338.

26- Debella P.B., S.R. Chavan,. 2008. RenapurkarLarvicidal activities of aqueous extracts of nineteen Indian plants against two mosquito species, *Aedes(s) Albopictus* and *Culex Quinquefasciatus* , Pesticides, 16 (2) , pp. 7–10

27- Stoll G. 2000. *Natural crop protection in the tropics*: Lettinginformation come to life. Margraf Verlag, Germany. 376. Pp.

28- Badshah, H., S.Z. Farmanullah., A.,Saljoqi, and M., Shakur, (2004). Toxic Effects of AK (*Calotropisprocera*) Plant Extracts Against Termites (*Heterotermesindicola* and *Coptotermesheimi*) Isoptera: Rhinotermitidae. *Pakistan Journal of BiologicalSciences*7: (9), 1603- 1606