IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

"Evalution of Anthelmintic Activity of Leaves Extract of Cardiospermum Halicacabum (L.)"

Manjusha Wath[1*], Kartiki Ingole[2]

[1]Associate Professor

[1]Department of Botany,

[1]Govt. Vidarbha Institute of Science and Humanities, Amravati, India

Abstract: *Cardiospermum halicacabum* (L.) is a climber which has been used for ages to relief from rheumatism, stiffness and snake bites etc. The plant reported as anti-inflammatory, anti-diuretic, anti-diabetic, anticarcinogenic, antibacterial, antiviral, anti-diarrheal, antioxidant, hepatoprotective and anthelmintic properties. The present study was aim to investigate the anthelmintic activity of plant *Cardiospermum halicacabum* (L.) and also to find out traditional alternatives of helminthiasis. According to their increasing polarity, the dried leaves of the plants were successively extracted by Soxhlet using distilled water as a solvent. *Pheretima posthuma*, an Indian earthworm, was treated by organic solvents such as distilled water, ethanol, and acetone at various concentration (50, 100, and 150 mg/ml). As a standard drug albendazole was used, while distilled water served as the control. The worm's paralysis and death time were determined. The presence of phytoconstituents such as alkaloids, tannins, flavonoids, and saponins, which are responsible for anthelminthic action, was revealed by a qualitative phytochemical analysis of the plant. In comparison to ethanol, distilled water, and albendazole, acetone extract from plants has strong anthelminthic activity while taking less time to paralyse and kill the earthworm.

Index Terms - *Cardiospermum halicacabum* (L.), *Pheretima posthuma*, Anthelmintic activity, Aqueous, Ethanol and Acetone extract.

I. INTRODUCTION

Germs live anywhere but all of them won't harm our body. Our immune system protects us against infectious agents. Infectious agents are bacteria, virus, fungi, protozoans and helminths which are highly infectious agents. Helminths are among the larger parasites. Hygienic conditions can reduce the infection at many extents. Recently corona virus pandemic, which has changed world scenario and also teaches us keeping hand clean helps to prevent spread of infection the same thing is with helminths. Along with hygiene herbal drugs who played important role in this regard. Plants have been used for therapeutic purpose since ancient time. According to WHO, approximately 65% of world population relied on plant derived traditional medicine for primary healthcare (Fransworth *et al*., 1985). Medicinal plants are an essential part of therapy in indigenous systems that have developed over ages in many cultures (Grunwald and Bruttel, 1996). Plant based drugs discovery and further innovative isolation methods play a great role in next generation drug discovery. Helminthiasis is one of the most common disease in humans and animals. Helminth infections affect a huge portion of global population (Gaikwad *et al*., 2011). Despite considerable improvements in treatment of parasites over the last few decades, there are currently no effective products to manage specific helminthiasis (Lateef *et al*., 2003). Helminth infections, known as helminthiasis, are parasitic worm illnesses of the human and animals. Worms normally only affect the gastrointestinal tract; however, they can sometimes affect other organs too (Mahadev *et al*., 2017). Helminthiasis poses a significant hazard to public health, including malnutrition, anemia, eosinophilia, and pneumonia. Anthelmintics are medications that strike or kill parasitic worms (helminths) and expel them from the body. It also affects animal population too. Because of the high cost of currently available anthelmintic medications, as well as the fact that gastrointestinal helminths become resistant to the drugs, helminth disease treatment is a major issue (Shelke *et al*., 2020). To get sustainable future the world is switching over herbal medicine and natural product (Kone *et al*., 2005). The plant kingdom is known to provide a rich source of phytoconstituents, which has been used as anthelmintic, antibacterial as well as insecticides activity. The present study was aim to investigate the "Anthelmintic Activity" of plant, *Cardiospermum halicacabum* (L.). To find out traditional alternatives to helminthiasis which can be both environmentally acceptable and sustainable. Such type of basic work could have more important role in future control of the helminth's infection.

II. MATERIAL AND METHODS

Work was carried out in three phases.

PHASE - I - Collection of the plant material

The leaves of *Cardiospermum halicacabum* (L.) were collected from the field area of Kathora Naka, Amravati, Maharashtra (India). Identification was done with the help of standard floras (Naik V.N., 1998 and Dhore M. A., 2002). The plant was identified and authenticated by Dr. Manjusha R. Wath, Associate Professor Govt. Vidarbha Institute of Science and Humanities, Amravati, Maharashtra. The voucher specimen was preserved in the Herbarium of Department of Botany. Herbarium specimen area prepared by following standard method. The leaves were thoroughly washed and then dried under shade for two weeks. The dried leaves were ground in a mixer grinder and sieved. The powder was stored in air sealed polythene bags at room temperature before extraction.

PHASE - II - Preparation of Extract

Soxhlet method:

Cardiospermum halicacabum (L.) powdered leaves (25 gm) were extracted using solvents in sequence of increasing polarity, i.e. water. The extraction was carried out for another 72 hours. Vacuum distillation was used to concentrate all of the extracts to dry mass once the extraction procedure was completed. The aqueous extract yielded 5.1 gm. The completed extracts were placed in an airtight container and preserved in the refrigerator until they were needed.

Preliminary phytochemical screening:

Phytochemical analysis was carried out with the help of following standard methods (Evans, 1997; Thimmaiah, 1999; Kulkarni and Apte, 2000). All the extracts were subjected to preliminary phytochemical screening for the presence or absence of various metabolites by following the standard procedures. The Qualitative tests for the presence of plant secondary metabolites such as alkaloids, phenols, tannins, flavonoids, saponins, steroids, fixed oils, lignins, terpenoids and glycosides were carried out.

PHASE - III - Anthelminthic Activity

- Procuring the worms –

Indian earth worms (*Pheretima posthuma*) were used as test worm in anthelmintic screening. The earthworms collected from Belsare organic farm, Amravati.

- Preparation of test sample and Experimental design –

Various concentrations (50,100,150 mg/ml) of each extract were prepared the bioassay. A total 10 ml for each concentration was prepared. Albendazole was used as a standard. Groups of approximately equal size worms consisting of 2 earthworms (*Pheretima posthuma*) individually in each group were releases into each 10 ml of desired concentration of the drug and extract in the petridish.

- Anthelmintic assay –

Aqueous, ethanol and acetone extract from the whole plant were investigated for their anthelmintic activity against *Pheretima posthuma*. Various concentrations (50,100,150 mg/ml) of each extract were tested in bioassay, which involved determination of time of paralysis and time of death of the worms. Albendazole were included as standard reference and distilled water as control. The anthelmintic assay was carried as per the method of with minor modifications. The assay was performed on adult Indian earthworms, *Pheretima posthuma* due to its anatomical and physiological resemblance. (Sollmann, 1918; Vidyarthi, 1967 and Thorn *et al*., 1977). Because of easy availability, *Pheretima posthuma* have been used widely for the evaluation of anthelmintic compounds in-vitro.

III. RESULT AND DISCUSSION

A) Macromorphology : *Cardiospermum halicacabum* (L.)

Family- Sapindaceae
Common name- Balloon Vine
Parts used- Leaves
Occurrence – Field area, Kathora Naka, Amravati.

Climber. Tap root. Herbaceous, aerial. Cylindrical, branched, fistular, hairy, dark brown. Cauline and ramal, opposite decussate, stipules, simple, ovate, parted acute, glabrous, reticulate, unicostate, membranous. Racemose, compound umbel. Bracteole, pedicel, pedicellate, complete, zygomorphic, hermaphrodite, pentamerous, vine colour. Sepals – 4, gamosepalous, induplicate-valvate. Petals – 4, gamopetalous valvate, Cruciform. Capsule, loculicidal, obovoid, papery brown, lobes elongated.

PHOTO PLATE – I

Cardiospermum halicacabum (L.)

B) Preliminary Phytoconstituents Analysis –

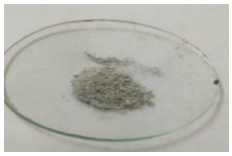
For preliminary Phytochemical screening of different extracts revealed the presence of different bioactive compounds by using different solvents such as ethanol and aqueous are presented in Table 1. The different methods carried out such as carbohydrates, alkaloids, tannins, flavonoids, saponins, proteins, amino acids, glycosides and terpenoids were found present while the phytochemical screening of alcoholic extract like carbohydrate, protein and tannin are found to be completely absent. All are found to be present in aqueous extracts.

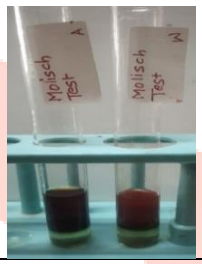
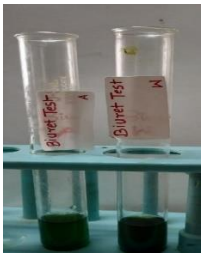
Carbohydrate, amino acids, alkaloids, glycosides, saponins, flavonoids and tannins are richly present compounds while Other metabolites like proteins and terpenoids are less richly present (Table 1).

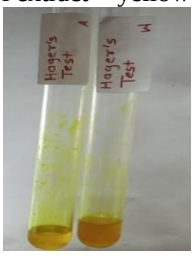
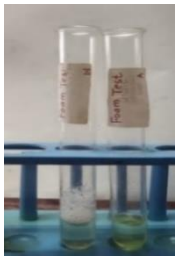
Table 1 – Preliminary phytochemical screening of *Cardiospermum halicacabum* (L.)

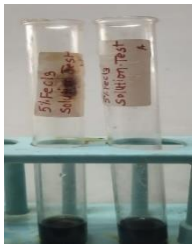
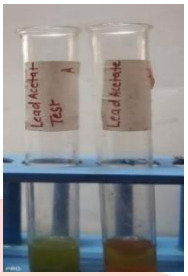
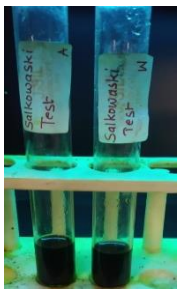
Test	Aqueous Extract	Alcohol Extract
Test for Carbohydrates		
Molisch test	+	-
Fehling's test	+	-
Test for Proteins		
Lead acetate test	+	-
Biuret test	-	-
Test for amino acids		
Ninhydrin test	+	+
Test for alkaloids		
Mayer's test	+	-
Hager's test	+	+
Wagner's test	+	-
Test for glycosides		
Legal test	+	+
Saponin foam test	+	-
Test for Flavonoids		
Shinoda test	+	+
Alkaline reagent test	+	+
Test for Tannins		
Ferric chloride test	+	-
Lead acetate test	+	-
Gelatin test	+	-
Test for Terpenoids		
Salkowaski test	-	+

Table 2 - Qualitative Analysis of *Cardiospermum halicacabum* (L.)

Sr. No.	Name of Test	Procedure	Observation	Result
1.	**Physical parameter**			
	• colour	Take a sample in petri dish and observe the colour.		Green in colour
	• odour	Take a sample in dish and smell	Pungent smell	Pungent odour
	• taste	Take a sample detect the taste	characteristics	Characteristic taste
2.	**standardization of sample**			
	• Loss on drying	• Weight about 2gm of coarsely powder of crude drug in crucible. • Initially weigh the empty crucible • Sample evaporate to dryness for 4 hr in hot air oven at 105^0c • Note the observation	1. Weight of powder sample taken (W1)=1.50gm 2. Weight of powder sample after drying taken(W2)= 1.41gm LOD = W1-W2 ÷ W2 ×100 =1.50gm – 1.41 ÷ 1.41 × 100 = 6.38 %	The loss on drying of drug is 6.38%. 
	• Ash value	a) Weigh and ignite flat, thin porcelain dish or tared silica crucible b) Weigh about 2gm of the powdered drug into dish/ crucible. c) Support the dish on pipe clay triangle placed on ring of retort stand d) Heat the flame, heat till vapours almost cease to be evolved then lower the dish and heat more strongly until all the carbon is burnt off e) Cool in desiccator Weigh the ash and calculate the percentage of total ash with reference to air dried sample of crude drug.	1. Weight of empty dish (x) = 73.33gm 2. Weight of dish and sample = 75.430 gm 3. Weight of sample taken(y) = 2.10 gm 4. Weight of the dish + Ash (after complete incineration) (z) = 75.415 gm 5. Weight of Ash obtained (z) = 0.085gm Total ash value = weight of ash ÷ weight of sample× 100 = 0.085÷ 2.10 ×100 = 4.04 % 	Total ash value obtained = 4.04%
	• pH	Drug taken 1gm Soluble in water	pH = 4.5 	slightly acidic
	• water soluble extractive	Drug taken - 5 gm. Solvent - 100 ml. pH - 4.5	Weight of extractive residue = 1.187 gm Percentage = 23.75 %	Percent = 23.75 %

				
	• Alcohol soluble extractive	Drug taken- 5 gm. Solvent- 100 ml. pH- 4.5.	Weight of extractive residue = 0.306 gm Percentage = 6.12 % 	Percent = 6.12%
3.	**Phytochemical testing**			
1.	**Test for carbohydrate** • **Molisch Test**	Powder sample + Molisch's reagent + H_2SO_4 from side of test tube.	Water extract = ring at junction Alcohol extract = No ring appear 	Water extract = carbohydrate present Alcohol extract carbohydrate absent
	• **Fehling's test (Reducing sugar)**	Powder sample extract + Fehling's solution A + Fehling's solution B + boil it for 2 min. A yellow red ppt observed	Water extract = red ppt Alcohol extract = no red ppt 	Water extract = carbohydrate present Alcohol extract = carbohydrate absent
2.	**Test for protein** • **Biuret test**	Sample extract 3ml + 4% NaOH and + few drop of 1% $CuSO_4$ solution. violet or pink colour appear	Water extract = No violet pink colour Alcohol extract = No violet pink colour 	Water extract = protein absent Alcohol extract = protein absent
	• **Lead acetate test**	2 ml test solution + 2 ml 40% NaOH + 0.5 ml lead acetate solution boil. Brownish Black colour appear.	Water extract = Brownish black colour Alcohol extract = No Brownish black colour	Water extract = Protein present Alcohol extract = Protein absent

3.	**Test for amino acid** • **Ninhydrin test**	Sample extract 3 ml + 3 drops 5% ninhydrin solution in boiling water bath for 10 min. purple or blue colour appear.	Water extract = Purple colour Alcohol extract = Purple colour	Water extract = Amino acid present Alcohol extract = Amino acid Present
4.	**Test for alkaloids** • **Mayer's test**	2 to 3 ml filtrate + Mayer's reagent. Cream colour ppt occurred.	Water extract = Cream ppt Alcohol extract = No Cream ppt	Water extract = Alkaloid present Alcohol extract = Alkaloid absent
	• **Hager's test**	2-3 ml filtrate + hagers reagent. Formation of yellow ppt.	Water extract = yellow ppt Alcohol extract = yellow ppt 	Water extract= alkaloid present Alcohol extract= Alkaloid present
	• **Wagner's test**	2-3 ml filtrate + 2-3 drops of Wagner's reagent. reddish brown ppt.	Water extract = reddish brown ppt Alcohol extract = no reddish ppt 	Water extract = alkaloid present Alcohol extract= alkaloid absent
5.	**Test for glycoside** • **Legal test**	Sample extract + 1ml of pyridine + 1ml of sodium nitroprusside. Pink to red colour appear	Water extract = Red colour Alcohol extract = Red colour 	Water extract = glycoside Present Alcohol extract = glycoside Present
	• **Saponin Foam test**	Shake the drug extract vigorously with water. persistent foam observed	Water extract = persistence foam Alcohol extract = no foam 	Water extract=saponin present Alcohol extract= saponin absent

6.	**Test for tannin and phenolic compound** • **Ferric chloride test**	Sample extract + 5% ferric chloride solution formation of deep blue black colour	Water extract = blue black colour Alcohol extract = No blue black colour 	Water extract = tannin present Alcohol extract = tannin absent
	• **Gelatine test**	Sample extract + 1% Gelatin + 10% NaCl. White ppt formed.	Water extract = White ppt Alcohol extract = No white ppt	Water extract = tannin present Alcohol extract = tannin absent
	• **Lead acetate solution test**	Sample extract + lead acetate solution. white colour ppt is formed	Water extract = white ppt Alcohol extract = No white ppt 	Water extract = tannin present Alcohol extract = tannin absent
7.	**Test for flavonoid** • **Shinoda test**	Sample extract + add 5ml 95% ethanol + drop of conc HCL +0.5 g magnesium turning. orange pink red colour appear	Water extract = orange colour Alcohol extract = orange colour	Water extract = flavonoid present Alcohol extract = flavonoid present
	• **Alkaline reagent test**	Sample extract + sodium hydroxide. Yellow colour occurred.	Water extract = Yellow ppt Alcohol extract = Yellow ppt	Water extract = Flavonoid present. Alcohol extract = flavonoid present
8.	**Test for terpenoid** • **Salkowski reaction**	Sample extract + Chloroform + Conc. H2SO4. Reddish Brown colour appeared.	Water extract = No Reddish Brown ppt. Alcohol extract = Reddish Brown ppt. 	Water extract = terpenoid absent Alcohol extract = terpenoid present

C) Anthelmintic Activity -

Cardiospermum halicacabum (L.) is a well-known medicinal plant and is widely used in folk ayurvedic system of medicine as a anthelmintic property. In the present study solvents namely ethanol, acetone and aqueous extract were used sequentially for crude extraction of *Cardiospermum halicacabum* (L.) plant leaves. To justify the ethnomedical claims of *Cardiospermum halicacabum* (L.) we made an efficient attempt in evaluating the anthelmintic property of *Cardiospermum halicacabum* (L.) with different concentrations.

PHOTO PLATE – II

Pheretima postuma in different concentration of extracts

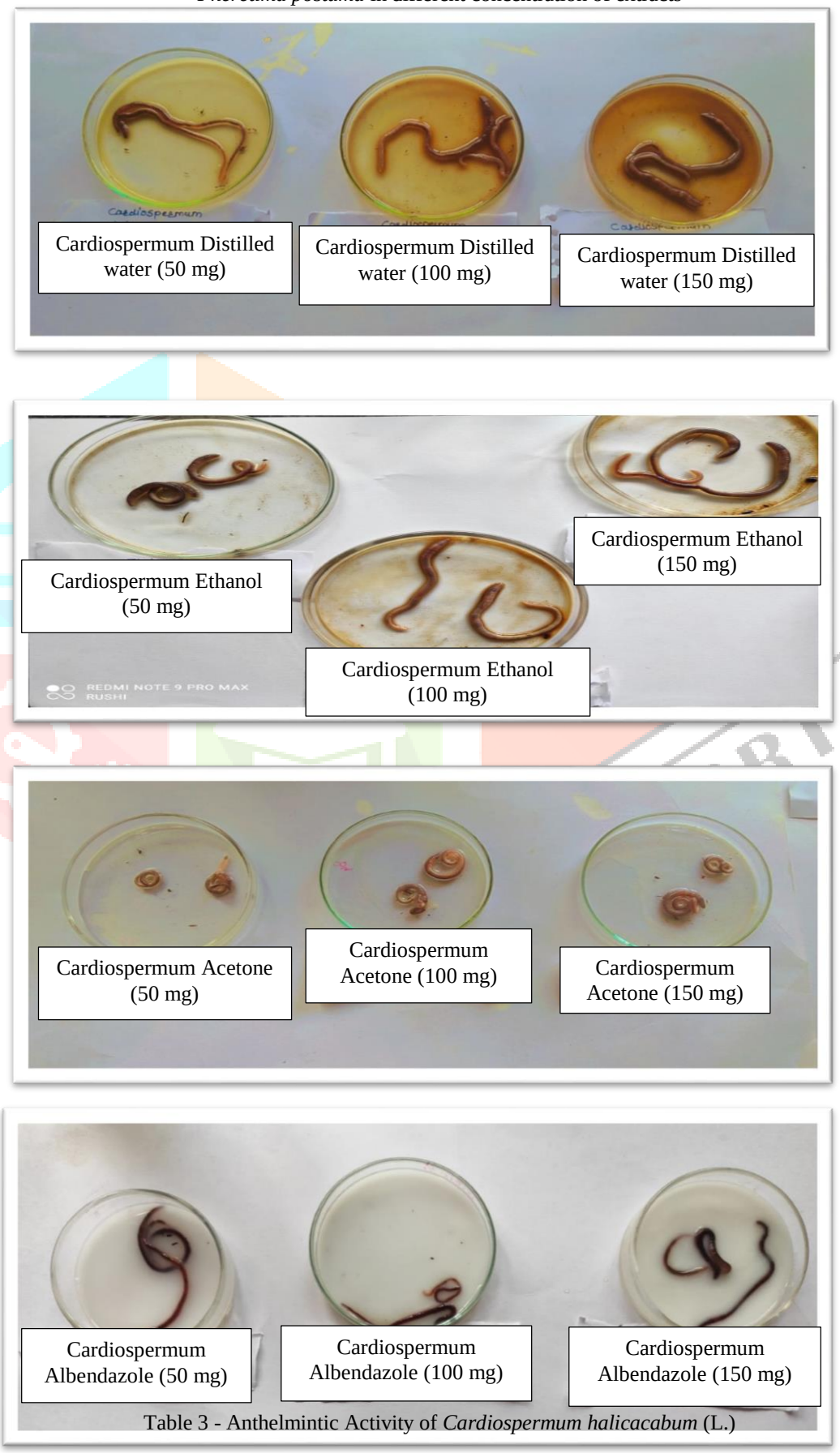


Table 3 - Anthelmintic Activity of *Cardiospermum halicacabum* (L.)

Test Sample	Concentration of Extract (mg/ml)	*P. posthuma* time taken for paralysis (In Min)	*P. posthuma* time taken for Death (In Min)
Aqueous	50	44	72
	100	31	64
	150	24	55
Ethanol	50	37	48
	100	25	37
	150	13	25
Acetone	50	25	39
	100	19	25
	150	12	21
Albendazole	50	25	32
	100	19	28
	150	17	22

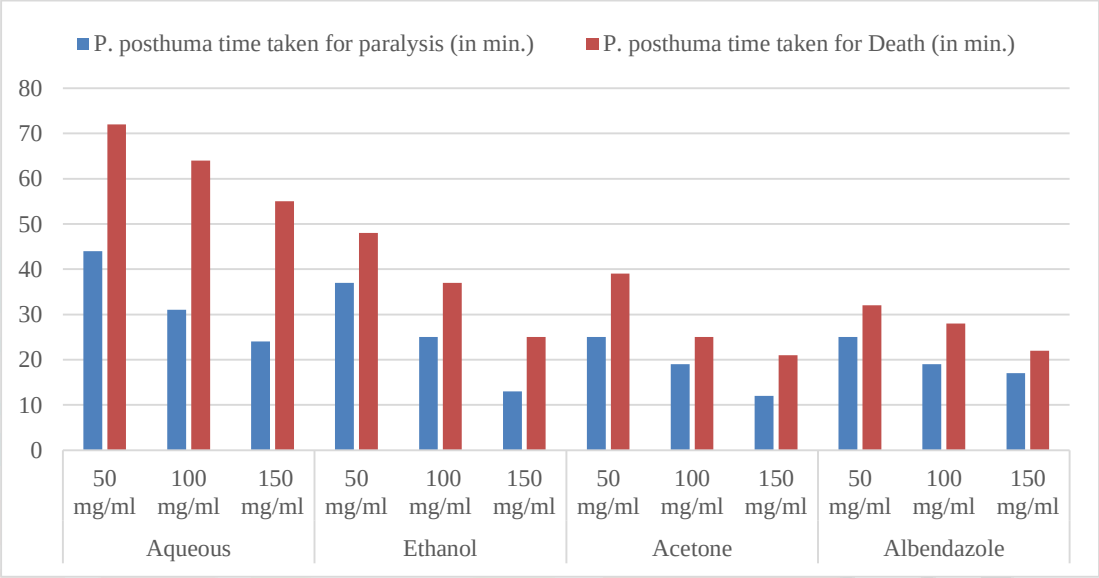


Fig. - 1. *Cardiospermum halicacabum* (L.) – showed the time for paralysis and death.

Table 3 and Fig. 1 showed that acetone extract of *Cardiospermum halicacabum* (L.) is significant anthelmintic activity against '***Pheretima posthuma***'. Acetone extract also proved to be efficient than the standard drug.

The **Acetone** extract of concentration **(50,100,150 mg/ml)** showed the paralysis time 25, 19, 12 min. and death time at 39, 25, 21 min. respectively. Acetone extract at 50 mg/ml showed efficient paralysis effect (25 min.) than other treated groups whereas acetone extract 50mg/ml showed significant anthelmintic activity with death time of (39 min.). Standard drug (50,100,150 mg/ml) showed paralysis time 25, 19,17 min and death time was 32,28,22 min. respectively. This investigation revealed that acetone extract of *Cardiospermum halicacabum* (L.) showed significant anthelmintic activity against *Pheretima posthuma* and also proved to be efficient than the standard drug.

The **Ethanol** extract of concentration **(50,100,150 mg/ml)** showed the paralysis time 37,25,13 min. and death time 48,37,25 min. respectively. Ethanol extract at 50 mg/ml showed efficient paralysis effect (37min.) than other treated groups whereas ethanol extract 50 mg/ml showed significant anthelmintic activity with death time of (48 min.). Standard drug (50,100,150 mg/ml) showed paralysis time 25,19,17 min and death time was 32,28,22 min. respectively. This investigation revealed that ethanol extract of *Cardiospermum halicacabum* (L.) showed less efficient anthelmintic activity against *Pheretima posthuma*. Ethanol is less efficient as compared to standard drug.

The **Aqueous** extract of concentration **(50,100,150 mg/ml)** showed the paralysis - time 44,31,24 min. and death time 72,64,55 min. respectively. Aqueous extract at 50 mg/ml showed efficient paralysis effect (44 min.) than other treated groups whereas aqueous extract 50 mg/ml showed significant anthelmintic activity with death time of (72 min.). Standard drug (50,100,150 mg/ml) showed paralysis time 25,19,17 min and death time was 32,28,22 min. respectively. This investigation revealed that aqueous extract of Cardiospermum halicacabun (L) showed significant anthelmintic activity against *Pheretima posthuma*. Aqueous extract is less efficient as compared to standard drug.

When **Albendazole** use as a standard drug extract of ***Cardiospermum halicacabum* (L.)** plant of concentration **(50,100,150 mg/ml)** showed **paralysis** at **25,19,17 min.** and **death** at **32,28,22 min.** Respectively.

From the above result, it is clear that the Acetone, Ethanol and Aqueous extracts of plant *Cardiospermum halicacabum* (L.) have significant anthelmintic activity in dose dependent manner when compared with standard anthelmintic drug. It reveals that the acetone extracts of *Cardiospermum halicacabum* (L.) plant took the less time to cause paralysis and death of the earthworm than that of acetone, ethanol, aqueous and albendazole standard drug.

(Aqueous extract > Ethanol > Acetone)

The phytochemical analysis of aqueous extracts of *Cardiospermum halicacabum* (L.) revels the presence of different compounds Carbohydrates, proteins, amino acids, alkaloids, glycosides, flavonoids, tannins and terpenoids. However, alcoholic was found positive for amino acids, alkaloids, glycosides, flavonoids and terpenoids only. These findings are partially in agreement with the findings of Kazmi *et al*., (1994). Daferera *et al*., (2003) found that these chemicals (table 1) which were found in significant amount having significant anthelmintic activity. Tannins are responsible for paralysis and death as it interfering with energy generation in helminths. It binds glycoproteins of cuticle responsible for death (Thompson and Geary, 1995) alkaloids also shown a potent activity on several helminth species. It specifically interferes with enzymatic systems of parasites. It also plays an important role in mobility of organism causing paralysis. Flavonoids have been reported to play a role in analgesic activity primarily by targeting prostaglandins (Rajnarayana *et al*., 2001). Alkaloids are well known for their ability to inhibit pain perception (Obianime *et al*., 2008).

The result is corroborated with the study of various workers in which they found that number of phytoconstituents responsible for anthelmintic activity (Kandagatla *et al*., 2019; Dillard *et al*., 2000).

In the present study crude powder and acetone extract showed 100% mortality. It could be due to synergistic effect of alkaloids, saponins, tannins, flavonoids. *Cardiospermum halicacabum* (L.) may be used as an alternative treatment of gastrointestinal helminthic in future.

IV. CONCLUSION

Anthelmintic study suggests that the fresh leaves of *Cardiospermum halicacabum* (L.) possess significant anthelmintic property. In the current study in vitro test of plant where perform. The extract of *Cardiospermum halicacabum* (L.) leaves showed a significant anthelmintic activity in dose dependent manner. In the light of the results present study, can be summarized the plant extract of *Cardiospermum halicacabum* (L.) possess several phytochemicals like alkaloids, tannin, flavonoids and saponins etc. which may be responsible for the possible anthelmintic activity. The present study concludes that acetone an extract of *Cardiospermum halicacabum* (L.) leaves possess significant anthelmintic activity against *Pheretima posthuma*. Further research is recommended to exploring the phytochemicals contents that was responsible for the anthelmintic activity from *Cardiospermum halicacabum* (L.). Furthermore, plants from different geographic areas should be evaluated using standard procedure as same plant in different soils have different chemical compositions may show different activities. These findings suggest that extracts from *Cardiospermum halicacabum* (L.) have promising anthelmintic effects.

V. REFERENCES

[1] Daferera, D.J., Ziogas, B.N. and Polissiou, M.G., (2003). The effectiveness of plant essential oils in the growth of Botrytis cinerea, Fusarium sp. and Clavibacter michiganensis subsp. michiganensis. Crop Protection, 22: 39-44.

[2] Dhore, M. A. (2002). Flora of Amravati district with special reference to the distribution of tree species. Amravati University Publication.

[3] Dillard, C. J., & German, J. B. (2000). Phytochemicals: nutraceuticals and human health. Journal of the Science of Food and Agriculture, 80(12), 1744-1756.

[4] Evans W. C., (1997). Trease and Evans Pharamcognosy. 14th Edition IV (B). Saunders Company Limited, Singapore.

[5] Fransworth, N. R., Akerele, O., Bingel, A. S., Soejarto, D. D., & Guo, Z. (1985). Drugs from medicinal plants. Bull. WHO, 63, 965-981.

[6] Gaikwad, S. A., Kale, A. A., Jadhav, B. G., Deshpande, N. R., & Salvekar, J. P. (2011). Anthelmintic activity of Cassia auriculata L. extracts-In vitro study. J. Nat. Prod. Plant Resour, 1(2), 62-66.

[7] Grunwald, J., Bruttel, K. (1996). The Europian phytotherapeutics market, Drugs made in Germany, 39: 6-11.

[8] Kandagatla, S., Arukala, M., and Mandapally, G. (2019). In-vitro evaluation of anthelmintic activity of aqueous extract of Nerium oleander. Journal of Pharmacognosy and Phytochemistry, 8(2), 1303-1305.

[9] Kazmi, M. H., A. Malik, Hameed, N., Akhtar and Noor Ali, S., (1994). Plant producta as antimicrobial agents. Phytochemistry, 36:761-763.

[10] Koné, W. M., Atindehou, K. K., Dossahoua, T., & Betschart, B. (2005). Anthelmintic activity of medicinal plants used in northern Côte d'Ivoire against intestinal helminthiasis. Pharmaceutical biology, 43(1), 72-78.

[11] Kulkarni, P. H., & Apte, B. K. (2000). Research Methodology for students of Ayurveda. Ayurveda Research Institute: Pune.

[12] Lateef, M., Iqbal, Z., Khan, M. N., Akhtar, M. S., & Jabbar, A. (2003). Anthelmintic activity of Adhatoda vesica roots. International journal of Agriculture and Biology, 5(1), 86-90.

[13] Mahadev, N. D., Thorat, A. T., & Vitthal, B. P. (2017). An evaluation of anthelmintic activity of Ricinus communis Linn. leaves by using different type of solvent. Journal of Pharmacognosy and Phytochemistry, 6(4), 1845-1847.

[14] Naik, V. N. (1998). Flora of Marathwada. Amrut Prakashan, Aurangabad, 1, 237-319.

[15] Obianime, A. W., & Uche, F. I. (2008). The Phytochemical screening and the effects of methanolic extract of Phyllanthus amarus leaf on the Biochemical parameters of Male guinea pigs. Journal of Applied Sciences and Environmental Management, 12(4).

[16] Rajnarayana K, Reddy M.S., Chaluvadi M.R., Krishna D.R. (2001). Biflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian J. Pharmacol., 33: 2.

[17] Shelke, P. S., Jagtap, P. N., & Tanpure, P. R. (2020). In-vitro anthelmintic activity of Boswellia serrata and Aloe barbadensis extracts on Pheretima posthuma: Indian earthworm. International Journal of Research in Medical Sciences, 8(5), 1843.

[18] Sollmann T (1918). Anthelmintics: Their efficiency as tested on earthworms. J Pharmacol Exp Ther 12: 129–170.

[19] Thimmaiah, S. K. (1999). Methods of biochemical analysis: carbohydrates. Standard methods of biochemical analysis. Kalyani publishers, Noida, 49-77.

[20] Thompson, D. P., & Geary, T. G. (1995). The structure and function of helminth surfaces. In Biochemistry and molecular biology of parasites (pp. 203-232). Academic press.

[21] Thorn, G.W., Adams, R.D., Braunwald, E., Isselbacher, K.J. and Petersdrof, R.G. (1977). Harrison's Principles of Internal Medicine. In: Mcgraw Hill Co., New York, pp. 1088-1089.

[22] Vidyarthi RD (1967). A Text Book of Zoology. S. Chand and Co., New Delhi. pp. 329 - 370.