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PHYTOCHEMICAL SCREENING AND *IN-VITRO* ANTHELMINTIC ACTIVITY OF SEED EXTRACTS OF PLANTS *APIUM LEPTOPHYLLUM* AND *APIUM GRAVEOLENS* OF FAMILY APIACEAE

S. M. Shiba[*1], G. Jeyabalan[2], A. K. Jha[3], M. Tapas[4]

[1*]Department of Pharmacy, JBIT, Dehradun, Uttarakhand, India.

[2]Department of Pharmacy, Sun Rise University, Alwar, Rajasthan 301001, India.

[3]SSTC-SSGI- Faculty of Pharmaceutical Sciences, Junwani, Bhilai, 490020CG, India.

[4]Department of Pharmacy, Kukreja Institute of Pharmaceutical Sciences, Jhajra, Dehradun, India.

ABSTRACT

The present research aimed at the *in-vitro* comparative study of the anthelmintic activity of aqueous and ethanolic extracts of seeds of *Apium leptophyllum* and *Apium graveolens* using Indian adult earthworms (*Pheretima Posthuma*) at 2 different concentrations (10 and 20mg/ml) in two different vol. 10ml and 15ml respectively. The study involved the determination of the time of paralysis (P) and the time of death (D) of the worms. At the concentration of 20mg/ml in both volume i.e. 10ml and 15ml the Ethanolic extracts showed very potent activities as compared to the standard drug Albendazole at two different concentrations (10 and 20mg/ml) volume taken 15ml. Ethanolic extract gives more potent result than aqueous extract when compare to standard. But aqueous extract also shows anthelmintic activity. In conclusion, aqueous and ethanolic extracts of seeds of *Apium leptophyllum* and *Apium graveolens* as an anthelmintic have been confirmed and further studies are suggested to discover the active principles responsible for the activity.

KEYWORDS

Apium leptophyllum, Apium graveolens, Anthelmintic, *Pheretima posthuma* and Albendazole.

Author for Correspondence:

Shiba S M,
Department of Pharmacy,
JBIT, Dehradun, Uttarakhand, India.

Email: shibamorris14@gmail.com

INTRODUCTION

The word Helminths is derived from the Greek word "helmins" which means "worm". Helminth is parasitic worms referring to various types of parasitic worms that live in the body[1]. Soil-transmitted helminths infection is the most common infection, many people worldwide suffer from this infection[2]. *Ascaris lumbricoides, Trichuris trichiura, Nacator amiricanus* and *Ancylostoma*

duodenal are the most common type of worms[3]. Most people affected by these infection lives in less developed countries of Africa, South America, and Asia, where deprivation , along with poor sanitary conditions, give rise to infections with intestinal helminth. Although not mortiferous in most cases, these parasites can cause considerable morbidities, such as anaemia and malnutrition, this leading to reduced growth and cognitive retardation, especially in children in endemic countries[4,5]. The World Health Organization disclose that over two billion people are suffering from parasitic worm infections[6] and it is estimated that by the year 2025, about 58% of the population in progressing countries will be influenced[7]. Effective treatments are desirable peoples affected by these parasites, the long-term efficacy remains undetermined and large-scale protective actions also bear the risk of resistances against the respective drugs[8-10]. This, in turn, will strongly limit the effective use of the very limited number of drugs against soil-transmitted helminths we are mainly depending on, namely albendazole, mebendazole, and levamisole[11]. At present the situation regarding resistances is not as severe as in veterinary medicine, monitoring of the drug efficacy should be improved and attempt in the development of new drugs be to speed up[12]. Natural products have always been a valuable source for the identification and the development of new drugs against various targets, helminth[6,13]. One access to discovering new drug is the investigation of plants based folk medicine on their traditional usage by an *in-vitro* verification of their respective bioactivity followed by advanced phytochemical studies leading to an isolation of the potent active principles[13]. The ubiquity of parasitic helminths typically displays a negative binomial distribution within an affected population such that relatively few persons carry heavy parasite burdens, without treatment, those peoples are most likely to become ill and to perpetuate infection within their community[14].

Anthelmintics are drugs that kill or expel worms from the GIT to eradicate adult helminths[15]. Helminth infections are now being conceded as the cause of many acute and chronic ill health among the human beings as well as cattle[16]. In most progressing and less progressed countries, helminth infections are a major health concern because they make humans prone to other infections such as microbial infections[17]. Intestinal infections with helminth can more easily treat than other infections that occur in other part in the body because the worms need to be eliminated by the drug and the drug need not be absorbed when given by oral route[18]. Most of the anthelmintics used today, produce side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhoea[19]. Anthelmintics from the natural sources may play an imp. Role in the treatment of these infections[20]. At least 80% of the world's population in developing countries uses plant materials as their source of primary health care[21]. Increasing problems of development of resistance in helminths against common anthelmintics have led to the proposal of investigate traditional medicinal plants for their anthelmintic activity[22]. Because of the increasing anthelmintic resistance and the impact of common anthelmintics on the environment, it is important to look for alternative approach against gastrointestinal nematodes[23]. Suitable targets in helminths include processes such as neuromuscular coordination, muscular activity, sensory processes, feeding and the regulation of coelomic pressure. There are some potent chemotherapeutic targets which include Energy metabolism, Nutrient uptake, Nucleic acid metabolism, anabolic pathways[24].

The Apiaceae is also known as carrot family, are a family of mostly plants with hollow stems. The family is large, more than three thousand seven hundred species spread across 434 genera. It is the sixteenth-largest family of flowering plants. Most Apiaceae are annual, biennial/ perennial herbs, the leaves aggregated toward the base, though a small species are shrubs or trees. Their leaves are of variable size and alternately arranged. The fruits are non-fleshy schizocarp of 2 mericarps, each with one single seed; they are split up at maturity and are dispersed by the wind. Fruit segments are covered in bristles and spread via extrinsic transport. The

seeds have an oily endosperm and generally contain large quantities of fatty oils, with the unsaturated fatty acid petroselinic acid occurring throughout the family while rarely being found outside of the Apiaceae

Apium leptophyllum Native- America, cultivated in India - Andhra Pradesh, Gujarat, Madhya Pradesh, and Karnataka. According to Ayurveda seeds of *Apium leptophyllum* also known as oregano in English are used as carminative, antimicrobial, anthelmintic, anti-inflammatory [rheumatic disorders, inflammation of the urinary tract], diuretic, nervine, sedative, antiemetic, antispasmodic, antiseptic also used in bronchitis, asthma, and liver and spleen diseases. Essential oil from seeds has tranquillizer, anticonvulsant, and antifungal effect. Seeds are also used in the treatment of chronic skin disorders[25,26].

Apium graveolen native to Europe mainly cultivated in the lowland of Italy from where it is spread to Sweden, Egypt, Algeria, Ethiopia, and Asia. In India, it is grown in north-western Himalayas, Punjab, Haryana, and in the hills of Uttar Pradesh, Himachal Pradesh, and southern India. According to Ayurveda and folk, medicine seeds of *Apium graveolens* are used as an aphrodisiac, antispasmodic, carminative, diuretic, emmenagogue, laxative, sedative, and stimulant. Celery is also known as mild diuretic and urinary antiseptic and has been in the relief of flatulence and griping pains. In the medicinal herbal market celery, oil or ground seed are touted as herbal that "promote and regulate" healthy blood pressure, joint health, and uric acid levels[27-28]. Root tinctures have been used as a diuretic in hypertension and urinary disorder[29]. Anti-inflammatory (used in rheumatic disorders, inflammation of the urinary tract), nervine, sedative, antiemetic, antiseptic also use in bronchitis, asthma and spleen diseases. Essential oil from seeds shows tranquillizer, anticonvulsant, antifungal and antimicrobial effect. Seeds are used in the treatment of skin disorders including psoriasis.

MATERIAL AND METHODS

Plant collection and Authentication

Apium leptophyllum and *Apium graveolens* are collected from local market of Chandigarh India and authenticated by Dr Anita Mahiswar, HOD, Department of Botany, Government Digvijay Autonomous PG College Rajnandgaon C.G.

Seeds are collected, air dried then reduced to coarse powder.

Preparation of Extract and Test sample

Aqueous extract

50gm coarse powdered drug was kept for maceration with 400ml of water for 72 hr. in a closed flask. The extract was filter by vacuum filtration. The filtrate was evaporated, the extract was dried and then used.

Ethanolic extract

50gm powder drug was kept for maceration with 400ml of Ethanol for 72 hr. The extract filter by vacuum filtration. The filtrate was evaporated in a rotary evaporator under reduced pressure until semi-solid extract was obtained, the extract was dried and then used.

The sample for the experiment was prepared by dissolving extract [Ethanolic and Aqueous extract]of each seed of plants *Apium leptophyllum* and *Apium graveolens* in 2% Tween 80 normal saline suspension to make the concentration of 10 and 20mg/ml and the volume was adjusted to 10ml and 15ml.

Animal

Pheretima posthuma were used to study the anthelminthic activity as these worms resemble anatomically and physiologically to the intestinal round worms[30-32]. Earthworm, *Pheretima posthuma*, are collected from moist soil and washed with normal saline solution to remove all faecal matter, the earthworms of 4-7cm in length and 0.1-0.2cm in width were used for experimental.

Reference standard and Chemicals

Standard Drug - Albendazole received from Ankur Drugs and Pharma Ltd. Unit 1 Manakpur, Solan [HP], Tween®80 [Molychem, Mumbai], Normal Saline [nirlife] purchased from the local shop.

Standard Albendazole was dissolved in 2% Tween 80 normal saline suspension to make conc. of 10 and 20mg/ml and the vol. was adjusted 15ml. Normal saline was taken as control.

Phytochemical screening

Phytochemical screening was carried out for all the extracts as per the standard methods and result is shown in Table No.1.

Alkaloid

Dragendorff's reagent

Alkaloid gives the reddish brown ppt.

Mayer's reagent

Alkaloid gives the cream colour precipitate.

Wagner's reagent

Alkaloids give the reddish brown ppt.

Hager's reagent

Alkaloid gives the yellow precipitate.

Tannic acid test

Alkaloids gives buff colour.

Amino acid

Millon's test

To the test solution adds about 2ml of Millon's reagent white precipitate indicates the presence of amino acid.

Ninhydrine Test

Add Ninhydrine solution to the test solution, boil violet colour indicates the presence of amino acid.

Proteins

Warming Test

Heat the solution over water bath, protein gets co-agulated.

Biuret test

To the test solution 2ml add 2ml biuret solution violet colour indicate the presence of protein.

Hydrolysis test

Hydrolyze the test solution with the HCl or sulphuric acid then carry out Ninhydrine test for amino acid.

Xanthoproteic test

To the 5ml of the test solution, add 1ml of conc. Nitric acid and boil yellow precipitate formed. After cooling it add 40% Sodium hydroxide solution, orange colour appears.

Starch

To the Aqueous extract adds week aqueous iodine solution blue colour indicate the presence of starch, which disappears on heating and reappears on cooling.

Steroids and Triterpenoids

Libermann-Burchard test

Treat the test extract with few drops of acetic anhydride boil then cool and add conc. Sulphuric acid by the side of test tube brown colour ring formed at the junction of two layers, the upper layer turn green which show the presence of Steroids, the formation of deep red colour show presence of Triterpenoids.

Salkowski test

Treat the solution with few drops of conc. Sulphuric acid red colour at the lower layer shows the presence of steroids and yellow colour lower layer indicate the presence of Triterpenoids.

Sulphur powder test

Add the small amount of sulphur powder to the test solution, Test sulphur powder sinks at the bottom.

Carbohydrates- Aqueous extract

Molisch's test

To the test sol. Add 2-3 drop of alcoholic a-naphthol then add 2-3 drop of conc. Sulphuric acid through the side of test tube purple to the violet colour ring formed at the junction.

Barfoed's reagent

1ml of test solution heated with 1ml of Barfoed's reagent on a water bath if the red cupric oxide is formed, show the presence of monosaccharide, Disaccharide on prolonging heating may reduce, owing to partial hydrolysis to monosaccharide.

Test for pentose

To the test solution adds an equal volume of hydrochloric acid containing the small amount of Phloroglucinol and heat red colour is appears.

Fehling's test

Filtrate were hydrolyse by using dil. HCl then neutralised with 5% solution of Sodium hydroxide and heat with Fehling's A and B solution formation of red colour precipitate indicate the presence of reducing sugar.

Glycoside

General test- Test A

Extract 200mg of the drug with 5ml of dil. Sulphuric acid by heating on water bath filter it then neutralized with 5% solution of NaOH and add 0.1ml of Fehling's A and B solution till it becomes alkaline, test with pH paper and heat on hot water bath for 2 min note the quantity of red colour ppt. form and compare with of form in test B.

Test B

Aqueous extract 200mg of the drug boil, and after boil add equal amount of water instead of 5% solution of NaOH used in test A Now add 0.1ml of Fehling's A and B sol till it become alkaline, test with pH paper and heat on boiling water bath for 2 min note the quantity of red colour ppt form and compare with precipitate form in test A. If precipitate of test A is more then test B then glycoside may be present. Since Test B shows the amount of reducing sugar in crude drug and test A represents free reducing sugar and those related on acid hydrolysis of any glycoside in the crude drug.

Anthraquinone glycoside

modified Bortrager's test- Boil 200mg of test material with 2ml of dil. Sulphuric acid treats it with 2ml of 5% aq. $FeCl_3$ solution freshly prepared, for 5 min, shake it with the equal volume of chloroform, separate the lower layer of chloroform and shake it with half of its volume of dil. Ammonia. A rose pink or red colour produced in Ammonical layer.

Test for Hydroxy-anthraquinone

Treat the test solution with potassium hydroxide solution red colour indicates the presence of Hydroxy- anthraquinone.

Cardiac Glycoside

Keller-Killiani test

Extract the drug with chloroform and evaporate it to the dryness. Add 0.4ml of glacial acetic acid containing the trace amount of Ferric Chloride. Transfers it to a small test tube add carefully 0.5ml of conc. Sulphuric acid by the side of test tube Acetic acid layer turns the blue colour.

Legal's test

To the test solution add pyridine and then add alkaline sodium nitroprusside solution red colour appear.

Baljet's test

Treat the solution with picric acid or sodium picrate orange colour is appears.

Cyanogenetic glycoside

Place 200mg of the drug in a conical flask and moisten with few drop of water. [There should be no free liquid in the flask as the test does not work because the hydrogen cyanide produce will dissolve in water rather than come out as a gas to react with the paper] moisten a piece of picric acid paper with 5% aqueous Sodium carbonate solution and suspend with the help of cork in the neck of flask. Warm gently at 39^oC, observe the color change. Hydro-cyanide is liberated from Cyanogenic glycoside by the enzyme activity and reacts with sodium picrate to form the reddish purple sodium isopurpurate.

Saponins

Froth test

Extract was dil, add distilled water upto 20ml and this was shaken in a graduated cylinder for 15 min formation of 1cm layer of foam indicate the presence of saponin.

Foam test

0.5mg of the extract was shaken with 2ml of water. If foam produces persist for 10 min. indicate the presence of saponin.

Flavonoid

Alkaline Reagent test

To the test sol. Add 2-3 drop of NaOH solution dark yellow colour appears which turn colourless an addition of few drop of dil. Acid HCl or H_2SO_4 indicate the presence of flavonoids.

Zinc hydrochloride test

To the test solution add zinc dust and conc. HCl, red colour appears in few minutes.

Diterpenes

The dissolve plant extract in water and treat with few drop of copper acetate solution formation of emerald colour show the presence of diterpenes.

Volatile oil

To the thin section of drug add Sudan III solution red colour obtain by globules show the presence of volatile oil.

Tannins and Phenolic compound

Gelatin test

To the test solution adds 1% gelatine solution containing 10% sodium chloride precipitate formed.

Ferric Chloride

Treat the test extract with $FeCl_3$ sol. blue colour appears if hydro stable tannins is present, Green colour appear if condense Tannins present.

Test for catechin

Dip the matchstick in the test sol. Dry it and then moisten with conc. HCl. Then warm the stick near flame the color of the wood changes to pink due to phloroglucinol is formed when catechin are treated with acid.

Test for chlorogenic acid

Treat the solution with aqueous ammonia and expose to air green colour is developed.

Naphthoquinones

Juglone test

To the chloroform extract add 2ml of ethyl ether with dil. Ammonia solution pink colour indicate Naphthoquinones.

Dam-Karrer test

To the chloroformed plant extract add 10% potassium hydroxide solution blue colour appears.

ANTHELMINTIC ACTIVITY

Anthelmintic activity of Ethanolic extract and Aqueous extract from the fruit commonly known as seeds of plants *Apium leptophyllum* and *Apium graveolens* were evaluated on Indian adult earthworms (*Pheretima posthuma*). Indian earthworms (*Pheretima posthuma*) were used to study the anthelminthic activity as these worms resemble both anatomically and physiologically to the intestinal roundworms[30-32], earthworms of 4-7cm in length and 0.1-0.2 cm in width were used for experimental. The earthworms were collected from moist soil and washed with normal saline solution, and are divided into the group of 7 each containing 5 earthworms for each extract of all seeds.

Extract were dissolved in 2% Tween 80 normal saline suspension to make the concentration of 10 and 20mg/ml and the volume was adjusted to 10ml and 15ml. Standard Albendazole was dissolved in 2% Tween 80 normal saline suspension to make the concentration of 10 and 20mg/ml and the vol. was adjusted 15ml. Normal saline was taken as control.

All the test sol. and standard drug sol. were prepared freshly before the performance of the experiment. Washed earthworm are put it in petri dish of different concentration [i.e. 10 and 20mg/ml] and different vol. [i.e. 10 and 15ml].

The observation value is observed in 5 observations. Five worms of about the same size in a petri dish were used. The time taken for complete paralysis and death of individual worms were noted. The time taken by worm to become complete motionless was noted as time of paralysis and time of death was when the worms lost their motility when dipped in the warm water [50°C] followed with fading away of their body color and by applying frequent outer stimuli which stimulate or induce movement in earthworm if alive. The mean of time of paralysis and time of death was recorded.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of *Apium leptophyllum* and *Apium graveolens* revealed the presence of Alkaloids, Amino Acid, Steroid, Glycoside, Cardiac glycoside, Saponin, Flavonoid, Tannin and phenolic compound (Table No.1). The anthelmintic activity of ethanolic extract of *Apium leptophyllum* and *Apium graveolens* was more potent than the aqueous extract of *Apium leptophyllum* and *Apium graveolens* was comparable with that of standard drugs Albendazole.

Anthelmintic activity of aqueous and ethanolic extracts of seeds of *Apium leptophyllum* and *Apium graveolens* using Indian adult earthworms (*Pheretima posthuma*) at two different concentrations (10 and 20mg/ml) in two different volumes 10ml and 15ml respectively. The study

involved the determination of time of paralysis and time of death of the worms. At the concentration of 20mg/ml in both volumes i.e. 10ml and 15ml the Ethanolic extracts showed very potent activities as compared to the standard drug Albendazole two different concentrations (10 and 20mg/ml) volume taken 15ml.

The extracts of *Apium leptophyllum* and *Apium graveolens* produced a significant anthelmintic activity in a dose-dependent manner as shown in (Table No.2 and Table No.3) and the graph shows (Figure No.1, Figure No.2, Figure No.3 and Figure No.4) Anthelmintic potential of both the extract with respect to Albendazole.

Discussion

In-vitro assays to screen the anthelmintic properties of plant extracts has main advantages that they are cheap and rapid result allow the screening of plants at large scale . In addition, these tests measured the effect of anthelmintic activity directly on the parasites without involving with the internal physiological functions of the host[33]. The higher activity of the ethanolic extracts as compared to the aqueous extract can be due to the presence of higher amounts of the phenolic compound as compared to aqueous extracts. The more useful explanation for the less activity of aqueous extract is due to the enzyme polyphenol oxidase, which degrades polyphenols in water extracts, whereas in ethanol they are inactive. Moreover, water is a better medium for the growth of the micro-organisms as compared to ethanol[34]. Higher concentrations of flavonoid compounds are seen in ethanol due to its higher polarity[35]. Also, ethanol was found easier to penetrate the cellular membrane to extract the intracellular compounds from the plant material[36]. Earthworms move by ciliary movement. The external layer of the *Pheretima posthuma* is mucilaginous and made up of complex polysaccharides. This layer being slippery, allow them to move freely. Any damage to mucopolysaccharide membrane will unveil the outer layer and this restricted its movement and can cause paralysis and this may lead to death of the worm[13,37]. All anthelmintics generally kill parasitic worms by either starving them to death or paralyzing them. As worms have no means of energy storing, they have to eat continuously to fulfil their metabolic needs. Any disruption in this process results in energy reduction and interfering with feeding for one day [24 hr] or less is sufficient to kill most adult parasites. If parasites become paralyzed and temporarily lose their ability to maintain their position in the gut this action will also lead to the death of parasites[38].

The possible mechanism of action of phytochemicals is- Tannins may interfere with energy generation by uncoupling oxidative phosphorylation[39-41]. Another possible mechanism of action is that they bind to free protein compound in the GIT of the host or to glycolproteins on the cuticle of the parasite worm and by this cause death. Alkaloids act on central nervous system of helminths and caused paralysis of the worm[42]. Steroidal alkaloid oligoglycosides may inhibit the movement of sucrose from the stomach to the small intestine which could diminish the support of glucose to helminth along with its antioxidant effect which is capable of decreasing the nitrate generation which is used in the protein synthesis as well as the possible inflammatory effect induced by the plant extract in the gastric and intestinal mucosal which could interfere in local homeostasis which is necessary for the development of helminthes[43]. The phytochemical screening of the extracts also shows the presence of saponins. Recent research addressed that the main biological activity due to saponins was their membrane permeabilizing property. The actions of action of saponin is changes in membrane permeability and pore formation, which is similar to the common anthelmintic drugs such as praziquantel. Saponin would affect the permeability of the cell membrane of the parasites and causes vacuolation and disintegration of monogenea teguments[44].

Coming to the chemistry of nematode outer surface, it is a collagen-rich extracellular matrix (ECM) providing defensive cuticle that forms an exoskeleton, and is critical for viability, the collagen is a class of protein that are modified by a

range co-translational and post-translational modification prior to assembly into the high-order complexes or collagen-rich extracellular matrix. The skin of mammals also consists mainly of collagen in the form of fibrous bundles. In leather making industry, plants tannins are generally used in the tanning operation of leather processing that imparts stability to collagen of skin matrix through its reactivity and hence makes the collagen molecule aggregate into fibres. This results in the loss of flexibility in the collagen matrix and gain of mechanical property with improved resistance to the thermal or microbial and enzymatic attacks. Same kind of reaction is expected to take place between the nematode or earthworm cuticle and the tannins of the plant extract, possibly by linking through hydrogen bonding. This form of reactivity brings toughness in the skin of worms and hence they become immobile and non-functional leading to paralysis followed by death[45]. The anthelmintic activities of crude extracts can also be explained in part by their partial lipophilic nature, which renders the cells leaky and thereby results in cell death[46].

The preliminary phytochemical screening of *Apium leptophyllum* and *Apium graveolens* revealed the b presence of Alkaloids, Amino Acid, Steroid, Carbohydrate, Glycoside, Cardiac glycoside, Saponin. Flavonoid, Tannin and phenolic compound. The anthelmintic activity of *Apium leptophyllum* and *Apium graveolens* might be due to the presence of alkaloids, tannins, saponins and phenolic compound. The possible mode of action for phytochemicals are-

Alkaloids

Which have an ability to Intercalates with the DNA synthesis of parasites[47] Acts on CNS and causes paralysis of worms[43]. Possess anti-oxidating effects, thus reduces nitrate generation which is useful for the synthesis of protein and hence interfering with local homeostasis of worms[48]. Transcuticular diffusion is a common means of transport to enter into helminth parasites[49] for non-nutrient and non-electrolyte compound in nematodes. It has also been shown that this route is predominant for the uptake of many broad spectrum anthelmintics by the different nematode, cestode and trematode parasites. The possible explanation for the better anthelmintic activity of ethanolic extract compared to aqueous extract on larvae and parasites could be due to easier transcuticular absorption[50].

Tannins

The anthelmintic effects of tannins may be due to its ability to bind free protein available in the tubes for larval nutrition and thus minimize nutrient availability could have resulted in the starvation of larval or decrease in gastrointestinal metabolism thus causing larval death. Increases the supply of digestible proteins by animals by forming protein complex compound in rumen. Binds to free proteins in G.I.T of host animal or cuticle of the parasite. Interferes with energy generation by uncoupling oxidative phosphorylation. React with the nematode. S cuticle and toughens the skin thus leading to paralysis. Tannin may Causes a decrease in G.I. metabolism resulting in secretion of mucous and chemicals harmful to the parasite[51,1].

Phenolic Compound

Interface with the energy generation and Uncoupling the oxidative phosphorylation thus Interfere with the glycoprotein of cell surface[52].

Saponins

Has membrane permeabilizing properties. Changes membrane permeability and pore formation of the cell membrane of parasites lead to vacuolization and disintegration of monogenea teguments[45].

Steroidal Alkaloid

Inhibits transfer of sucrose from the stomach to small intestine, thus stop the support of glucose to the helminths[44].

The possible explanation for the better activity of the alcoholic extract compared to the aqueous extract on parasitic worms in the current study could be due to easier transcuticular absorption of the alcoholic extracts into the body of the worm than the aqueous extracts. In general, alcoholic extracts of plants contain some non-polar organic chemicals with less polarity than the aqueous extracts, rendering them more lipid soluble than the aqueous extracts and hence shows better anthelmintic activity. Lipophilic anthelmintics

agent has a more capability to pass the outer surface of the helminths than the hydrophilic compounds[53]. Albendazole is broad spectrum oral anthelmintic its mechanism of action - inhibiting microtubule synthesis thus irreversibly damaged glucose uptake, as a result, intestinal parasites are immobilised or die slowly[54].

Table No.1: Show results of phytochemical screening of *Apium Lyptophyllum* and *Apium graveolens*

S.No	Test	*Apium lyptophyllum*		*Apium graveolens*	
		Aq.	**E**	**Aq.**	**E**
ALKALOID					
1	Dragendorff's reagent	-	+	-	+
2	Mayer's reagent	-	+	-	+
3	Wagner's reagent	+	+	-	-
4	Hager's reagent	+	+	-	-
5	Tannic Acid Test	+	+	+	+
AMINO ACID					
6	Millon's Test	**+**	**+**	**+**	**+**
7	Ninhydrine Test	**+**	**+**	**+**	**+**
PROTEIN					
8	Warming Test	**-**	**-**	**-**	**-**
9	Biuret Test	**-**	**-**	**-**	**-**
10	Hydrolysis Test	**-**	**-**	**-**	**-**
11	Xanthoproteic Test	**-**	**-**	**-**	**-**
STARCH		**-**	**-**	**-**	**-**
STEROID AND TRITERPENOIDS					
12	Libermann-Burchard Test	+	+	+	+
13	Salkowski Test	+ yellow	+ yellow	+ yellow	+ yellow
14	Sulfur Powder Test	+	+	+	+
CARBOHYDRATE AQUEOUS EXTRACT ONLY					
15	Molish Test	**+**	**-**	**+**	**-**
16	Barford Test	**+**	**-**	**+**	**-**
17	Test For Pentose	**+**	**-**	**+**	**-**
18	Fehling's Test	**+**	**-**	**+**	**-**
GLYCOSIDE					
19	General Test	**+**	**+**	**+**	**+**
20	Modified Borntrager Test	**+**	**+**	**+**	**+**
21	Test for Hydroxy-Anthraquinone	**-**	**-**	**-**	**-**
CARDIAC GLYCOSIDE					
22	Keller Killiani Test	**+**	**+**	**+**	**+**
23	Legals Test	**+**	**+**	**+**	**+**
24	Baljets Test	**+**	**+**	**+**	**+**
CYNOGENETIC GLYCOSIDE		**-**	**-**	**-**	**-**

SAPONIN					
25	Forth Test	**+**	**+**	**+**	**+**
26	Fome	**+**	**+**	**+**	**+**
FLAVONOID					
27	Alkaline Reagent Test	**+**	**+**	**+**	**+**
28	Zinc Hydro-cholride Test	**+**	**+**	**+**	**+**
DITERPENS		**-**	**-**	**-**	**-**
VOLATILE OIL		**+**		**+**	
TANNINS AND PHENOLIC COMPOUND					
29	Gelatine Test	+	+	+	+
30	Ferric Chloride Test	+ Green	+ Green	+ Green	+ Green
31	Test for catechin	**-**	**-**	**-**	**-**
32	Test for chlorogenic acid	**+**	**+**	**-**	**-**
NAPHTHOQUINONES					
33	Juglone Test	**-**	**-**	**-**	**-**
34	Dam-Karrer Test	**-**	**-**	**-**	**-**

Table No.2: Result of Anthelmintic activity of aqueous extract

S.No	Plant Name	Aqueous Extract				Aqueous Extract			
		10mg/ml volume taken 10ml		10mg/ml volume taken 15ml		20mg/ml volume taken 10ml		20mg/ml volume taken 15ml	
		Time of paralysis	Time of Death	Time of paralysis	Time of Death	Time of paralysis	Time of Death	Time of paralysis	Time of Death
1	*Apium lyptophyllum*	62.0312 ±0.424814	84.4782 ±0.070793	60.18 ±0.099907	81.4492 ±0.045664	52.4146 ±0.1182	72.5246 ±0.041555	51.3076 ±0.169717	71.0136 ±0.251634
2	*Apium graveolens*	48.8934 ±0.374543	78.2962 ±0.226616	47.2268 ±0.167765	76.345 ±0.250351	35.1382 ±0.155611	49.138 0.142104	33.3722 ±0.157455	46.4906 ±0.066286
3	Standard Albendazole			11.2872 ±0.221321	27.305 ±0.252536			7.1942 ±0.1187	22.4684 ±0.116991

Table No.3: Result of Anthelmintic activity of Ethanolic extract

S.No	Plant Name	Ethanolic Extract				Ethanolic Extract			
		10mg/ml volume taken 10ml		10mg/ml volume taken 15ml		20mg/ml volume taken 10ml		20mg/ml volume taken 15ml	
		Time of paralysis	Time of Death	Time of paralysis	Time of Death	Time of paralysis	Time of Death	Time of paralysis	Time of Death
1	*Apium lyptophyllum*	6.313 ±0.074515	19.8564 ±0.282593	5.0212 ±0.26014	18.272 ±0.071725	4.4846 ±0.086147	17.4248 ±0.168024	3.7472 ±0.282087	16.1832 ±0.160774
2	*Apium graveolens*	8.6582 ±0.237188	23.3636 ±0.117866	7.4134 ±0.137602	21.2266 ±0.119339	5.3384 ±0.119178	19.225 ±0.143496	4.3272 ±0.227468	17.2652 ±0.205761
3	Standard Albendazole			11.2872 ±0.221321	27.305 ±0.252536			7.1942 ±0.1187	22.4684 ±0.116991

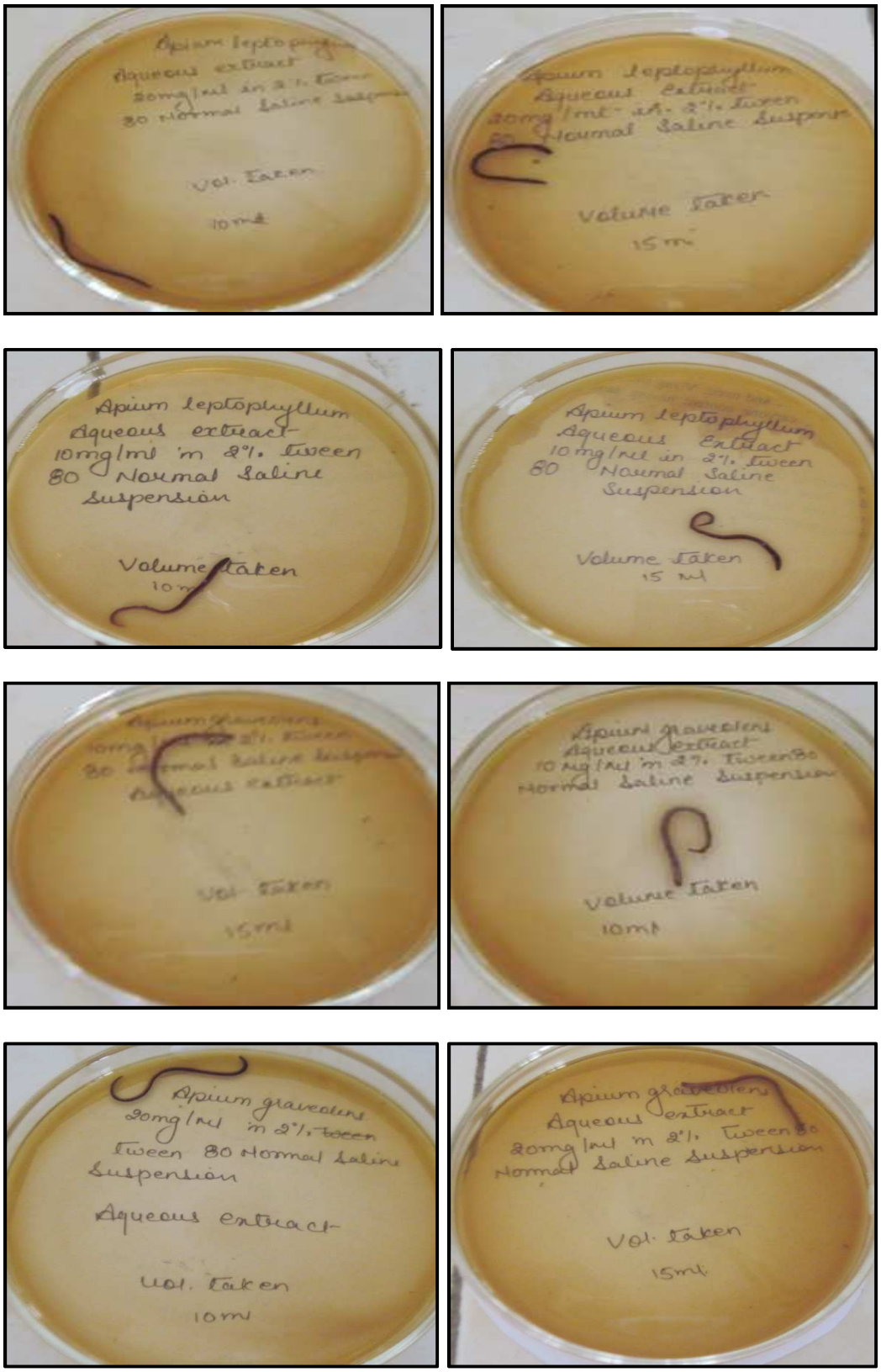

Figure No.1: Anthelmintic activity of aqueous extract of *Apium lyptophyllum* and *Apium graveolens*

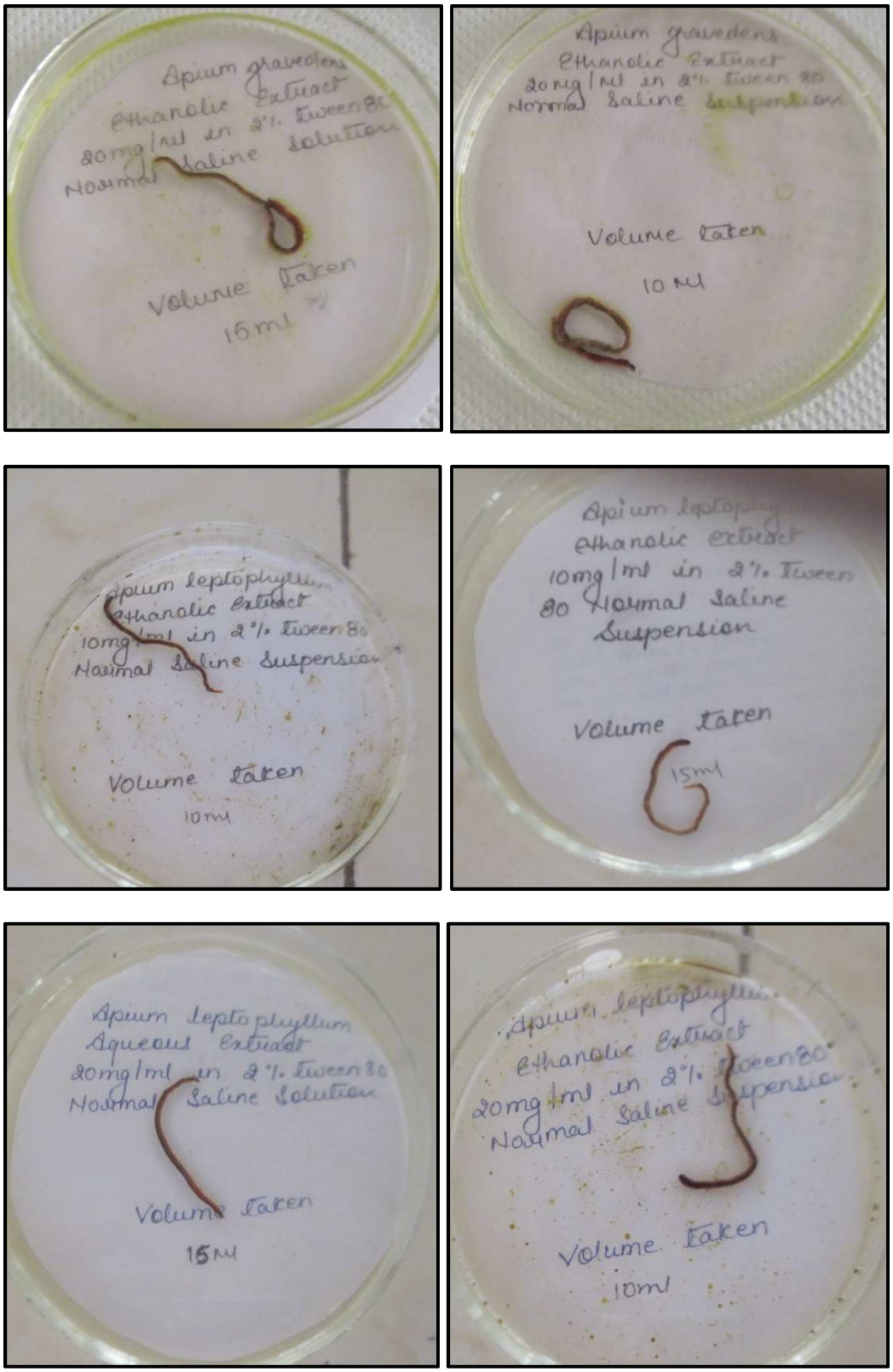

Figure No.2: Shows Anthelmintic activity of Ethanolic extract of *Apium lyptophyllum* and *Apium graveolens*

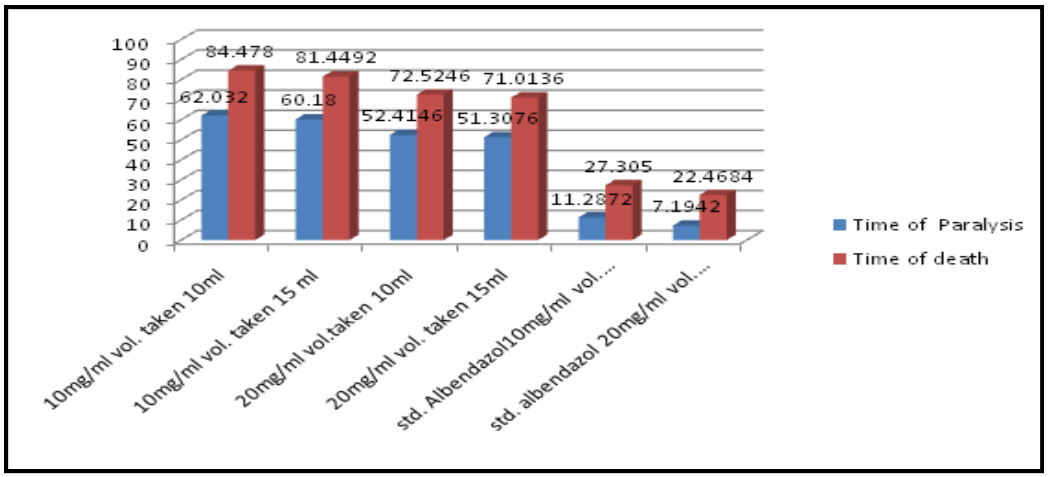

Figure No.3: Time of paralysis and Time of death of *Pheretima posthuma* by Water extract of *Apium lyptophyllum* compare to std. Albendazole

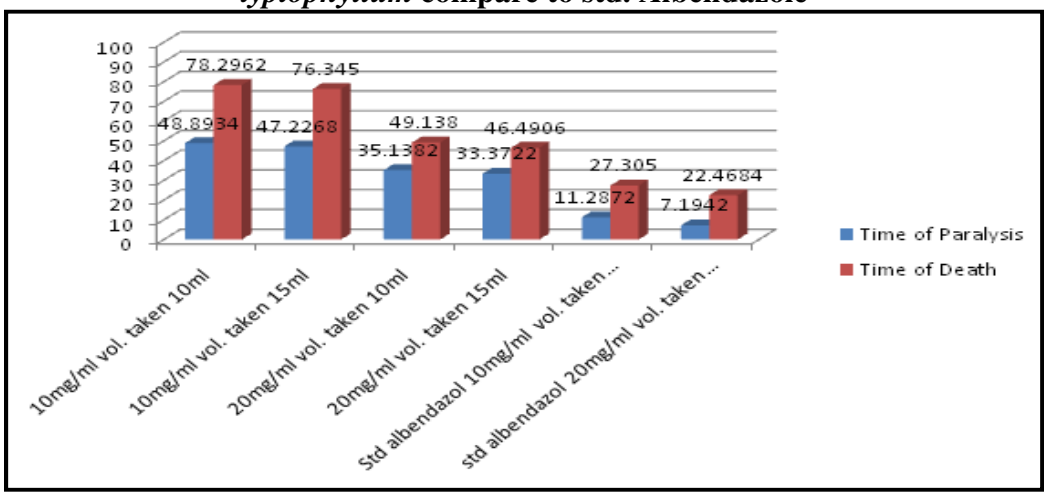

Figure No.4: Time of paralysis and Time of death of *Pheretima posthuma* by water extract of *Apium graveolens* compare to std. Albendazole

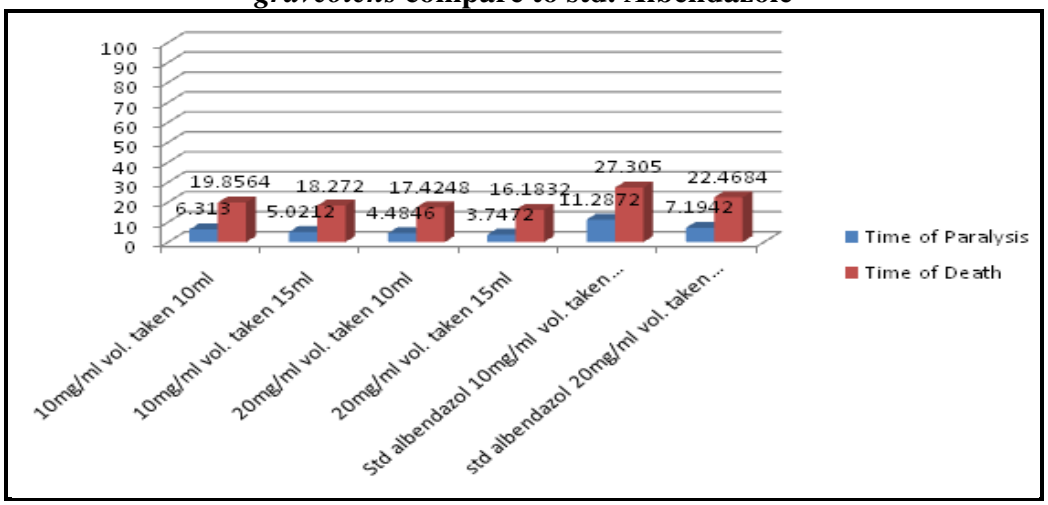

Figure No.5: Time of paralysis and time of death of *Pheretima posthuma* by Ethanolic extract of *Apium lyptophyllum* compare to std. Albendazole

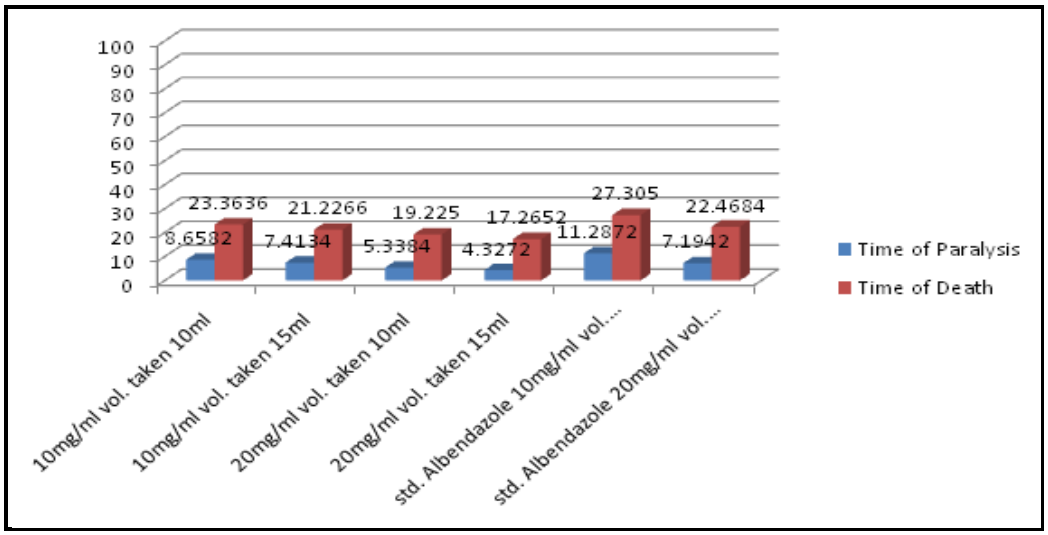

Figure No.6: Time of paralysis and time of death of *Pheretima posthuma* by Ethanolic extract of *Apium graveolens* compare to std. Albendazole

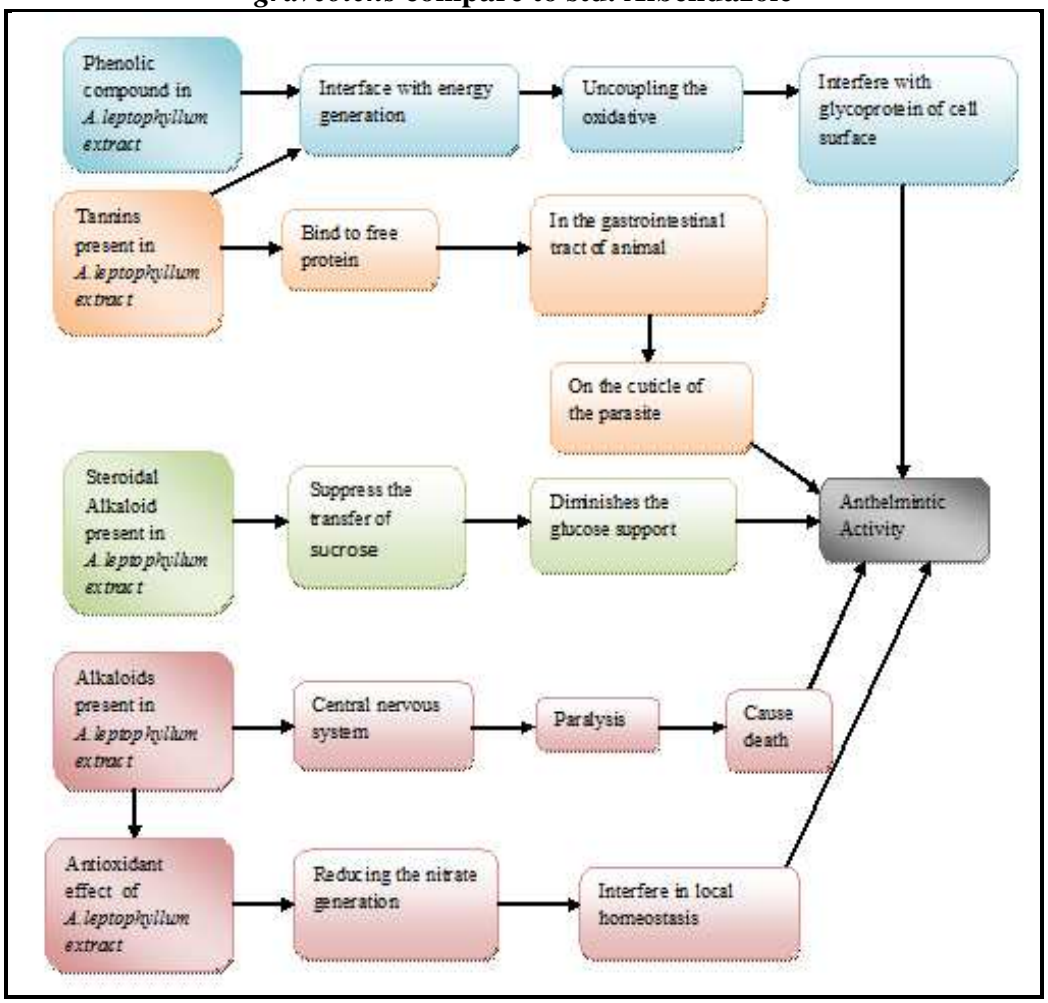

Figure No.7: Possible mechanism of Action of Phytochemical present in the extract of *A. leptophyllum* as Anthelmintics

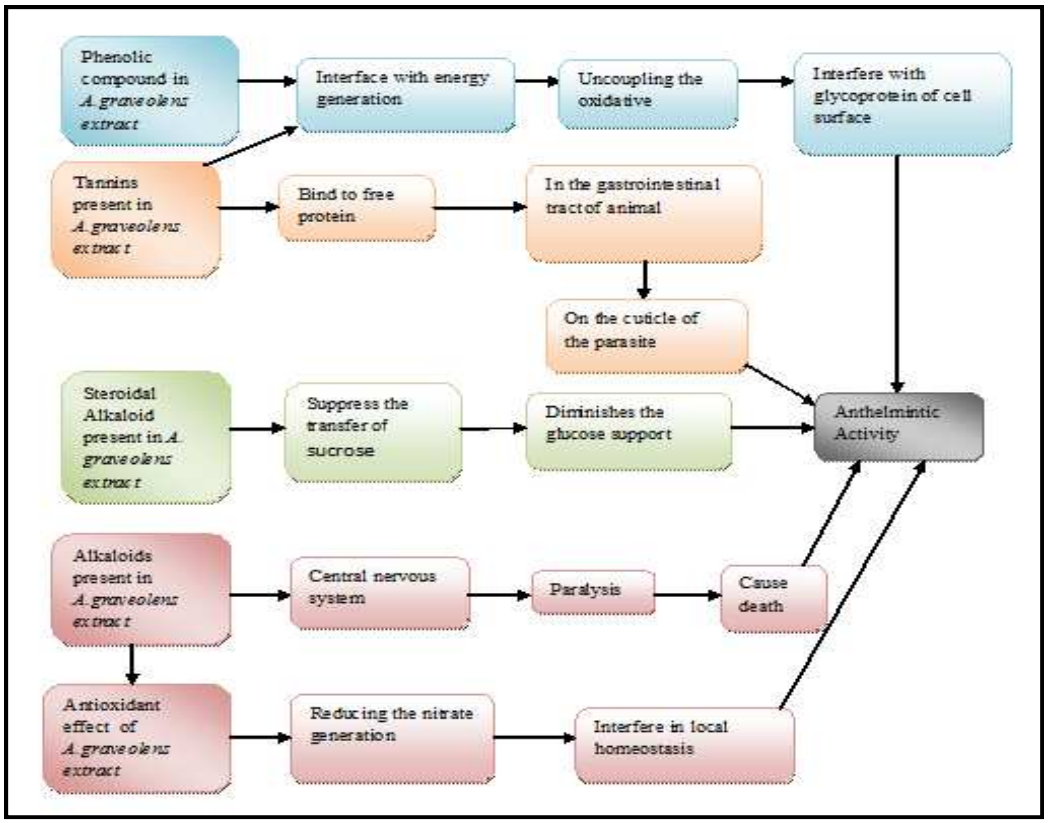

Figure No.8: Possible mechanism of Action of Phytochemical present in the extract of *A. graveolens* as Anthelmintics

CONCLUSION

It is concluded that active constituents responsible for anthelmintic activity are present in the aqueous and ethanolic extracts of seeds of *Apium leptophyllum* and *Apium graveolens*. Further work will emphasise the isolation and characterization of active principles responsible for the anthelmintic activity of seeds extracts of *Apium leptophyllum* and *Apium graveolens*.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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