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Screening of antihelmintic activity of methanol, petroleum ether and silver nanoparticles of insulin plant *Costus igneus* N. E. Bar.

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Abstract: *The aim of the present study was to screen Antihelmintic activities of methanol, petroleum ether and silver nano particles of insulin leaf extract. The work was carried on the different concentration levels as o.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/ml of both extracts and insulin leaf silver nano particles with water and Albendazole control. The finding showed that anthelmintic activity by methanol, petroleum ether and insulin leaf silver nano particle activity was successfully. The helminthic activity was observed in order to insulin leaf silver nanoparticle > methanol extract > petroleum ether. The results were compared with the standard drug, Albendazole and it was found that insulin leaf silver nano particles were more effective than the standard drug and extracts. It has been used mostly for research purposes and monitoring and evaluating control programs of anthelmintic activity in future.*

Key Words: *Antihelmintic activity, methanol, petroleum ether, Insulin leaf silver nano particles.*

INTRODUCTION:

Insulin plant, *Costus igneus* (Nak) [syn. (*Costus pictus* (D. Don), *Costus mexicanus* (Liebm ex Petersen) or *Costus congenitus* (Rowle)], commonly known as Fiery Costus, Step ladder or Spiral flag or Insulin plant. Its leaves help to build up insulin in the human body [1]. It is an oral hypoglycemic agent and controlled blood glucose level [1]. Literature also showed various activities such as, hyperglycemic [2], antiproliferative [3], hyperlipidemic [4], diuretic [5], antimicrobial [6], antiurolithiatic [7], anti-inflammatory [8], learning and memory [9], antioxidant [10], ameliorative [11], anticancer [12], putative [13], and neuroprotective [14]. According to the available literature on the pharmacological and phytochemical prospective of *Costus speciosus*, no any reports are available on the anthelmintic activity of the any extracts of the insulin plant. Based on this, an attempt has been made to evaluate the anthelmintic activity of the methanolic and aqueous extracts of the aerial parts of the plant. However, in the present study the insulin plant solvent extract of silver nano particles was desired for the work. The insulin plant silver nanoparticle study was not reported by any of the workers hens, the study was desired. Thus, these herbal medicines hold a great scope for not only new drug discoveries against parasitic diseases but also for further exploration for scientific evidence regarding the treatment and control of intestinal helminthiasis.

Helminthiasis, or worm infestation, is one of the most prevalent disease and one of the most serious public health problems in the world. Helminths affect more than 2 billion people worldwide and cause a range of adverse health problems, including anaemia, diarrhoea, and abdominal pain, impaired cognitive and physical development, particularly in developing countries [15]. Among the most widespread of all chronic infections are those caused by various species of parasitic helminthes. For example, it is estimated that over half the world's population may be infected with gastrointestinal helminthes. Inhabitants of tropical or subtropical low-income countries are most at risk; children often become infected with one or more species. Almost 350 species of helminthes have been found in humans, and most colonies the gastrointestinal tract. In some cases, these infections result mainly in discomfort and do not cause substantial ill health, but others, such as schistosomiasis and hookworm disease, can produce very serious morbidity. Because of its prevalence, the problem of the treatment of helminthiasis is therefore one of very great practical therapeutic importance [16].

MATERIALS AND METHOD:

Plant Material: Fresh aerial parts of *Costus igneus* N. E. Bar. (Costaceae) for the proposed work were collected from the P. V. P. College, Biotechnology Garden, Loni, located at 740 35'-37'N latitude and 190 24-28'E longitude, District

Ahmednagar, MS, India. The plant was identified, authenticated and a voucher specimen was kept in herbarium.

Preparation of Extracts: The dried and coarsely powdered aerial parts of the plant were used for the extraction. The coarse powder of the plant was successively extracted using a Soxhlet apparatus with the solvents in increasing polarity starting with petroleum ether, methanol and water. The extracts of the aerial parts of *Costus igneus*were concentrated by rotary evaporator at 40°C under reduced pressure and then dried and stored in desiccators for future use. The dried extracts were suspended in 0.5% CMC in distilled water (vehicle) and used for anthelmintic activity (Table 1).

Preparation of Ag-nanoparticles using aniline: The silver sol was prepared by reduction of Ag+ ions using addition of ethyl alcohol containing 2 % PVP solution with weight ratios of $AgNO_3$ metal salt to PVP being 1:5. The glass rod was sonicated in a sonic bath and the prepared suspension will be applied to a polyethylene substrate manually using controlled wire-wound rod. The Ag film looks very uniform over the entire substrate. The same procedure was followed to prepare silver sol for synthesize nano-film on glass substrate that previously was immersed in the sol of as prepared coating material at a constant uniform speed. After 24 hours substrate was pulled up with same uniform speed.

Experimental Specimen: Indian adult earthworms, *P. posthuma* were collected from moist soil of the field and washed with tap water to remove all fecal matter for further investigation. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were selected for the experiment. The anthelmintic activity was evaluated on adult Indian earthworm (*P. posthuma*) due to its anatomical and physiological resemblance with the intestinal round worm of human beings and also easy availability, earthworms have been used widely for initial evaluation of anthelmintic compounds.

Study protocol: In the study purpose five groups with water and Albendazole standard control were designed in triplicate. Approximately equal size and weight worms of ten individuals were used for the present study. The concentrations were made as 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/ml for each one extracts and data recorded (Table 1).

Anthelmintic Activity: The anthelmintic assay was carried out as per the method [17] with minor modifications. Different extracts and the standard drug solution were poured in different petri dishes. All the earthworms of approximately equal size after washing were released into 10 ml of respective solutions of different concentrations. All the earthworms were divided into five groups with 10 earth worms in each group. The first group serves as control receives only 0.5% CMC in distilled water, the second group serves as standard receives Albendazole (10 mg/ml) suspended in 0.5% CMC and the remaining groups receive 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/ml, concentrations of insulin leaf silver nano particles. The corresponding concentration was expressed in term of mg of extract per ml of solvent (mg/ml). The drug and extract solutions were freshly prepared before starting the experiment. The anthelmintic potency of the extracts was inversely proportional to the time taken for paralysis or death of the worms. The standard drug solution and the extracts caused paralysis followed by death of all selected worms at the selected concentrations. The time taken to paralyze and kill individual worms was observed. The time of paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. The times of death of the worms were recorded after ascertaining that worms neither moved when shaken vigorously or when dipped in warm water (50°C). Death was concluded when the worms lose their motility followed with fading away of their body colour. All the results were shown in Table 1 and expressed as a mean SEM of three replica in each group.

Statistical analysis: The data on biological studies were reported as mean ± S. E. M.. The analysis of variance (ANOVA) at 5 % level significant was employed. $P \leq 0.05$ were considered significant.

DISCUSSION:

In the observations were made for the time taken to paralysis and death in individual worms. The death was concluded when the worms lost their entire activity followed with fading away of their body colour. Time of paralysis was noted down when no movement of any worm observed. The time of death for worm was recorded after ascertaining that the worms were neither moved nor vigorously moved when deepen in warm (50^0C) water. The paralysis time and death time were recorded in term of minutes.

An effect of albendazole on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole by increasing chloride ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis [18]. It is observed that methanolic petroleum extracts and silver nano particle of *C. igneus* showed excellent anthelmintic activity at all the concentrations. The methanolic and petroleum ether extract showed more significant effect on paralyzing the worms, in terms of paralysis time, at every concentration compared to that of methanolic extract when compared with standard.

Helminthic infections of the gastrointestinal tract of human beings and animals have been recognized to have adverse effects on health and lowering of resistance. In search of compounds with anthelmintic activity, a number of substances were screened using different species of worms, for example, earthworms, Ascaris, Nippostrongylus, and Heterakis. Of all these species, earthworms have been used widely for the initial evaluation of anthelmintic compounds because they resemble intestinal worms in their reaction to antihelmintics. It has been demonstrated that all

antihelmintics are toxic to earthworms and a substance toxic to earthworms is worthy for investigation as an Anthelmintic [19]. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols, etc. The extracts of plant have revealed the presence of alkaloids, flavonoids, cardiac glycosides, saponins, sterols and tannins [20]. From the above results, it is concluded that the extracts of the plant have potent anthelmintic activity when compared with the conventionally used drugs and is equipotent to standard anthelmintic drug. The anthelmintic activity was performed on same sized, aged and weight of earthworm. The species was selected for anthelmintic activity because of anatomical and physiological resemblances with the intestine of round worm parasites of human beings [21].

The purpose of this study is to evaluate and compare the anthelmintic activity of the methanolic, petroleum ether and insulin silver nanoparticles extracts of the leaf parts of *Costus igneus* in earthworms (*P. posthuma*). The anthelmintic potency of the extracts was inversely proportional to the time taken for paralysis or death of the worms. As *Costus igneus* showed significant anthelmintic activity in the experimental study, it can be used as a promising anthelmintic agent. All the results were expressed as a mean±SEM. As *Costus igneus* showed significant anthelmintic activity in the experimental study, it can be used as a promising anthelmintic agent.

Previous worker reported *Ipomoea carnea* plant leaf was sequentially extracted as ethanol, methanol, petroleum ether and chloroform as the solvent system. The trends of activity were in order as chloroform > methanol > ethanol > petroleum ether. The anthelmintic activity may be due to the presence of polyphenol compounds [22]. The leaf of Ipomoea carnea contained thirteen bioactive compounds in which any one should have been an anthelmintic property. Hence, there is need to find out fractionation of leaf contents of *Ipomoea cornea* [23].Quashri *et al*, [24] reported best cytological and anthelmintic activity from methanolic extracts of *Parthenium hysterophorus*. Herbal drugs have been used and best effective and could be of value of preventing the development of drug resistance. They noticed moderate number of alkaloids, flavonoid, saponin, and tannin [25] polyphenol [26] reflecting anthelmintic activity. Phenolic and tannin compound show anthelmintic activity by binding to glycoprotein on the cuticle of the parasite and thus lead to death of the worm [27].

RESULT:

The result is presented in this section. In case of the methanolic extract at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/ml concentrations paralysis were observed at 2.5±0.38, 3.0±0.41, 3.0±0.51, 4.8±0.38, 4.6±0.31, and 5.6±0.30 min and death at 14.5±0.42, 15.0±0.37, 15.5±0.38, 16.0±0.27, 16.5±0.31 and 17.0±0.39 min post-exposure respectively. The Petroleum ether extract 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/ml concentrations paralysis were observed 9.5±0.40, 9.0± 0.42, 8.5±0.35, 8.0±0.19, 7.5± 0.51 and 7.0±0.47 respectively while death was observed within 17.9± 0.29, 18.2 ± 0.40, 19.7 ± 0.24, 23.0 ± 0.29, 24.7 ± 0.33 and 25.0 ± 0.39 respectively. The insulin silver nanoparticles extract 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/ml concentrations paralysis were observed 2.5 ± 0.41, 3.0 ± 0.33, 3.5 ± 0.31, 4.0 ± 0.36, 4.2 ± 0.41and 4.4 ± 0.35 respectively while death was observed within 3.5 ± 0.47, 4.2 ± 0.41, 4.7 ± 0.28, 5.2 ± 0.38, 5.7 ± 0.42, and 6.2 ± 0.44 respectively. The standard drug Albendazole (10 mg/ml) showed paralysis at 4.2 ± 0.28 min and death occurred after 9.3 ± 0.33 min. The earthworms were more sensitive to the extracts of *C.igneus* as compared to the reference drug albendazole (10 mg/ml). The results were compared with the standard drug, Albendazole and it was found that both extracts were more effective than the selected standard drug. The helminthic activity was insulin silver nanoparticle > methanol extract >petroleum ether. It showed that insulin leaf silver nanoparticles are good helminthic activity than other two tested extracts.

CONCLUSION:

The present study showed the potential anthelmintic activity of *Costus igneus* leaf parts. The plant may have different mode of actions against the selected helminthes. The helminthic activity trend was insulin silver nanoparticle > methanol >petroleum ether. The possible mechanism of the anthelmintic activity of *Costus igneus* cannot be explained on the basis of our present results. The plant may be further explored for its phytochemical profile to recognize the active constituent and standardization of dose and toxicity studies for drug development accountable for anthelmintic activity.

CONFLICT OF INTEREST: Authors have declared that no competing interests exist. The study performed in this research is commonly and predominantly used for study.

Table 1. Anthelmintic activity of methanol, petroleum ether and insulin silver nanoparticles.

Sr. No	Samples	Concentration (mg/ml)	Time required (Minutes)	
			Paralyzed	Death X±S.E.M.

			X±S.E.M.	
1	Water	10	-	-
2	Albendazole	10	4.2 ± 0.28	9.3 ± 0.33
3	Methanolic Extract	0.5	2.5 ± 0.38	14.5 ± 0.42
		1.0	3.0 ± 0.41	15.0 ± 0.37
		1.5	3.0 ± 0.51	15.5 ± 0.38
		2.0	4.8 ± 0.38	16.0 ± 0.27
		2.5	4.6 ± 0.31	16.5 ± 0.31
		3.0	5.6 ± 0.30	17.0 ± 0.39
4	Petroleum ether	0.5	9.5 ± 0.40	17.9± 0.29
		1.0	9.0 ± 0.42	18.2 ± 0.40
		1.5	8.5 ± 0.35	19.7 ± 0.24
		2.0	8.0 ± 0.19	23.0 ± 0.29
		2.5	7.5 ± 0.51	24.7 ± 0.33
		3.0	7.0 ± 0.47	25.0 ± 0.39
5	Silver Nanoparticles	0.5	2.5 ± 0.41	3.5 ± 0.47
		1.0	3.0 ± 0.33	4.2 ± 0.41
		1.5	3.5 ± 0.31	4.7 ± 0.28
		2.0	4.0 ± 0.36	5.2 ± 0.38
		2.5	4.2 ± 0.41	5.7 ± 0.42
		3.0	4.4 ± 0.35	6.2 ± 0.44

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