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Anthelmintic Efficacy Of Gerbera Gossypina (Royle) Beauverd Against Gastrointestinal Nematodes Of Sheep

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Abstract:
Background*: The study was conducted to investigate phytochemical studies and the anthelmintic property of Gerbera gossypina (Royle) Beauverd, locally named as (Patejhhulo), and tested by in vivo and in vitro experiments against gastrointestinal nematodes of sheep from April to November 2022. G. gossypina indicated the content of flavonoids, glycosides, carbohydrates phenolic compounds, alkaloids terpenoid and steroids, while negative for saponins, anthocynin.*
Materials and Methods*: In vivo studies sheep were randomly divided into five treatment groups with six animals in each group and reared in similar environmental and managemental condition in same shed. Among five treatment group, positive and negative control groups received Albendazole (ABZ) and No Treatment (NT) respectively; remaining three groups received different doses of ethanol extract of G. gossypina as T1: 150 mg/kg body weight, T2: 200 mg/kg body weight and T3: 250 mg/kg body weight. Fecal egg count reduction (FECR) was calculated on day 0, 7, 14, 21 and 28. Data were analysed using SPSS (version 25.0) software for statistical analysis of mean, significantly different means were compared using Ducan's Multiple Range Test (DMRT) computer software package For in vitro test, egg hatch test was performed with different doses of ethanol extract of G. gossypina ie. 50 mg/ml, 25 mg/ml, 12 mg/ml and 6 mg/ml respectively. Data were analyzed by Least square procedure using Harvey (1990) computer software and DMRT ($p<0.001$) was considered for separately/comparing the means which were significant among treatment.*
Results*: The in vivo results indicated that EPG was significantly reduced by T2 and T3 on day 21 and day 28, as compared to NT group, which is similar to that of ABZ. The best EPG loads reduction was found on 28 days by T3 (250 mg/ml). The highest reduction rate on FEC of treated group was 89.79% on 28-day post- treatment at dose rate 250mg/kg body weight. In vitro result indicated that ethanol extract at 50 mg/ml exhibit significant ($p<0.001$) ovicidal activity against eggs of GINS compared to distilled water. Ethanol extract showed dose dependent inhibition in in vitro egg hatch assay.*
Conclusion: *Therefore, quality-controlled extracts of G. gossypina whole plants or possibly isolated bioactive compounds could be promising alternatives to conventional anthelmintics for current anthelmintics used to treat gastrointestinal nematodes in small ruminants.*
Key Word*: Gerbera gossypina, anthelmintic, phytoscreening test, in-vitro test*

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I. Introduction

Sheep are susceptible to helminth infection, which can lead to serious economic losses, especially when it is brought on by deficient feeding, poor management, and insufficient anthelminthic medications (Risso *et al.*, 2015). The most common diseases in the sheep herd were nematodiasis (54.0%), followed by contagious ecthyma (33.3%), scabies (28%), and pneumonia (12%) (Shrestha & Karmacharya, 2016). Numerous parasitic nematodes with veterinary importance have genetic traits that encourage the development of anthelmintic resistance, which has developed into a significant global challenge to the production of small ruminants. The emergence of anthelmintic resistance poses severe effects on both future sheep productivity and the welfare of grazing sheep. Different species of gastrointestinal nematodes have developed varying degrees of resistance to all the major classes of anthelmintic drugs (Papadopoulos, 2008).

The rapid development of resistance to commonly used drugs among nematodes, associated with high cost, environmental pollution and food residues have given new interest in medicinal plants as an alternative source of anthelmintic drugs. Nepalese farmers are using various types of plants as medicinal plants in remote areas, of which *Gerbera gossypina* (*Patejhhulo*) is considered one of them. Only one information about the plant was described

by Gyawali, R. R., & Paudel, H. R. (2017). But no data is available regarding the study on the medicinal use of this *Gerbera gossypina* (*Patejhhulo*) plant or plant extract to reduce the internal parasitic load in small ruminants or its efficacy and effect on animal health. That is why; this study was carried out to investigate the anthelmintic efficacy of *Gerbera gossypina* (*Patejhhulo*) against gastrointestinal nematodes of sheep.

II. Material And Methods

A preliminary personal communication with the farmers of Jumla (Western Nepal) was done at the earlier period of the research during February. Most commonly used plant with anthelmintic property was selected and among the group of plants *Gerbera gossypina* with such property was selected. From April to July 2022, the fresh complete plant of *Gerbera gossypina* was harvested from its natural habitat near Kankasundari Gaupalika Ward No. 5 Lankhu, Jumla, Nepal. Collection No. HA001 for *Gerbera gossypina* was placed at the National Herbarium of Godawari, Lalitpur, following botanical identification and authentication of the collected plants

Study Location: Livestock farm at Agriculture and Forestry University (AFU), Chitwan (Nepal) from March to November 2022.

Study Duration: March 2022 to November 2022.

Sample size: 60 sheep's

Sample size calculation:

Sheep naturally infected with gastrointestinal nematodes were screened by using direct smear method, fecal floatation method as described by Soulsby (1982) and Eggs per Gram (EPG) was calculated using modified Mc Masters technique. Altogether 30 sheep having EPG at least 100, were chosen and randomly divided into five groups, each comprising 6 animals.

Subjects & selection method:

The Sheep that were reared in semi-intensive management system (grazed for 6-8 hours/day) were selected. Out of 82 sheep raised on the farm, 60 sheep (3-7 months to 2 years age group) were included in the experiment due to the uneven age group and case of pregnancies. The animals sampled for the study had not received any anthelmintic medications in the 2 months before because the experiment could involve pre-selected larvae and not the general population. In the sheep flocks, the estimation of body weight for correct dosing of extract and treatments was done by weighing the largest and smallest sheep by a digital weighing balance and the weight of the other remaining sheep was visually estimated compared with the largest and smallest.

Procedure methodology

Fresh whole plant of *Gerbera gossypina* was collected and ethanol extract of plants was made for the treatment in respective group of sheep.

Table 1. Treatment with different groups of animals (In-vivo)

Group	Treatments	Route	Dosage (mg/kg)
T1	*Gerbera gossypina*	Os	150
T2	*Gerbera gossypina*	Os	200
T3	*Gerbera gossypina*	Os	250
ABZ	Albendazole	Os	10
NT	Not treatment (NT)		

At first, fecal samples were collected from all the sheep allocated in different groups at day 0 just prior to the administration of drugs (pretreatment). After that, fecal samples were collected on 7, 14, 21 and 28 days after the treatment at predetermined intervals and evaluation of anthelmintic activity of the ethanol extract of *Gerbera* under *in vivo* conditions against gastrointestinal nematodes of sheep was done by using standardized protocol of World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles et al., 1992), which was conducted in laboratory of Department of Pharmacology and Department of Parasitology, AFU, Rampur.

Faecal egg percent reduction was calculated using the formula of the following:

$$\textbf{FECR (\%)} = \frac{\boldsymbol{Pre\ treatment\ EPG - Post\ treatment\ EPG} \times \mathbf{100}}{\boldsymbol{pretreatment\ EPG}}$$

For *in vitro* test, egg hatch inhibition test was done. Total 30 fecal samples were divided into 6 groups each group consists of five replications. In this assay, in each well approximately 100 µl of egg suspension with approximately 200 eggs was pipetted in 96 well microtiter plates. These fecal sample were tested with different doses of ethanol extract of *G. gossypina* ie. 50 mg/ml, 25 mg/ml, 12 mg/ml and 6 mg/ml respectively and incubated at 27 ˚C for 48 hours and number of larvae were counted under microscope.

Egg hatch inhibition percent was calculated by the formula of following:

$$\textbf{Egg hatch inhibition} = \frac{\boldsymbol{Egg\ per\ gram\ in\ suspnsion - larva\ count}}{\boldsymbol{egg\ per\ gram\ in\ suspension}} \times \mathbf{100}$$

Table 2. Treatment with different groups of animals (In-vitro)

Group	Treatments	Concentration (mg/ml)
T1	*Gerbera gossypina*	6
T2	*Gerbera gossypina*	12
T3	*Gerbera gossypina*	25
T4	*Gerbera gossypina*	50
ABZ	Albendazole	25
NT	Not treatment (NT)	

Statistical analysis

Using SPSS, the data were statistically evaluated using ANOVA. By applying ANOVA, the differences among the means were analyzed. Afterward, as a post hoc test, the Duncan test for homogeneity of variance was done to separate the means (SPSS, 2019) in one-way ANOVA.

III. Result

(A) In vivo effect of anthelmintic activity of ethanol extract of *Gerbera gossypina*

Effect on fecal egg count per gram (EPG)

The EPG from day 0 pre-treatment to day 28 of *Gerbera gossypina* treated sheep in the form of ethanol extract at dosage rate of 150 mg/kg (T1), 200 mg/kg (T2) and 250 mg/kg (T3) BW as compared with ABZ at dose 10 mg/kg BW treated with positive control and Non-treatment (NT) negative control is shown in Table 3. Initial day, the EPG loads were found significant different among treatments and control group by 5 % level. An average EPG load was found 4633.33 which was higher in T4 (5583.33) and lowest was found in T3 (4250). The EPG loads were reduced in 14 days, 21days and 28 days by treatments and control group at 1 % level of significant. The best EPG loads reduction was found in 28-day interval among T3 and T4.

Table 3. Egg per gram (EPG in 100) from day 0 to day 28 of *Gerbera gossypina* treated sheep as compared with non-treatment (NT) negative control and albendazole (ABZ) treated positive control

	T1 (n= 6) [1]	T2 (n=6) [2]	T3 (n=6) [3]	T4 (n=6) [4]	T5 (n=6)[5]	Overall (n=30)	F Value	P – value
0 day	4733.33[ab] (799.16)	4633.33 [a] (668.33)	4250.00 [a] (686.29)	5583.33 [b] (661.56)	3966.66 [a] (920.14)	4633.33 (894.94)	3.971**	0.013
7 days	4416.66 [ab] (1045.78)	3641.66 [ab] (1371.28)	3183.33 [a] (801.04)	4928.33 [b] (977.65)	4358.33 [ab] (1190.13)	4105.66 (1194.23)	2.383	0.078
14 days	3291.66 [ac] (854.64)	2933.33 [a] (1269.12)	1225 [a] (672.12)	2450 [ab] (1556.92)	4458.33 [c] (1117.32)	2871.66 (1506.86)	6.481***	0.001
21 days	2433.33 [a] (411.90)	1558.33 [ab] (835.71)	583.33 [b] (470.81)	950.00 [b] (447.21)	4850.00 [c] (1385.27)	2075.00 (1717.69)	27.082***	0.000
28 days	1816.66 [a] (328.12)	1416.66 [ab] (435.50)	458.33 [b] (490.32)	596.66 [b] (631.02)	5233.33 [c] (1419.38)	1904.33 (1907.56)	38.455***	0.000

Note: Figures in parentheses indicate standard deviation. *** and ** resemble significance at 1% and 5% levels, respectively.

[1] T1 @150 mg/kg BW

[1] T2 @200mg/kg BW

[1] T3 @250 mg/kg BW
[1]T4 ABZ @10 mg/kg BW
[1] T5 @Negative Control

Effect on fecal egg count reduction (FECR)

FECR from day 7 to day 21 *Gerbera* treated sheep in form of extract at dose 150 mg/kg BW (T1), 200 mg/kg BW (T2), 250 mg/kg BW (T3) as compared with Albendazole (ABZ) at dose 10 mg/kg BW as positive control and Non treatment as negative control is Table 4. The treatment wise comparison revealed that on day 7, T3 decreased FEC significantly ($p<0.01$) comparison with NT while insignificant ($p<0.01$) compared to ABZ. On 14 days, also T3 decreased FECR significantly ($p<0.01$) comparison with NT, which was comparable to effect of ABZ. Similarly, on 21st day, T2 and T3 were comparable with the effects of the ABZ. Comparing the treatment on day 28 after treatment, T2 with average reduction 68.10% and T3 with average reduction 89.79% substantially different from ($p<0.01$) NT, while reduction by T2 was insignificant compared to ABZ, while T3 was significant compared to ABZ at 1 % level of significant. The average reduction in FEC for the treated group ranged from 25.47%, 63.39%, and 78.89% on 7th, 14th, and 21st day following treatment, respectively. The largest decrease in FEC for the treated group was 89.79% on 28th day after treatment at a dosage amount of 250 mg/kg BW. On 7th, 14th, 21st and 28th day following treatment, the average FEC reduction rate in 150 mg/kg BW was 18.53%, 30.90%, 47.99%, and 59.82%, respectively.

Table 4. FECR from day 7 to day 28 of *Gerbera gossypina* treated sheep as compared with non-treatment (NT) negative control and albendazole (ABZ) treated positive control

Treatments	No. of samples	7 days	14 days	21 days	28 days
T1	6	18.53^{a}	30.90^{a}	47.99^{a}	59.82^{a}
T2	6	21.41^{a}	36.30^{ab}	65.95^{b}	68.10^{a}
T3	6	25.47^{a}	63.39^{c}	78.89^{b}	89.79^{b}
ABZ	6	27.20^{a}	53.54b^{c}	75.65^{b}	89.34^{b}
NT control	6	-9.25^{b}	-12.20^{d}	-21.67^{c}	-31.26^{c}
Overall	30	16.67	34.39	49.36	55.16
F value		5.557***	17.025***	63.275***	105.130***
P value		0.002	0.000	0.000	0.000

Note: *** indicates significant at 1% level

(B) In-vitro effect of ethanol extract of gerbera gossypina (Royle) Beauverd on egg hatch test/assays

The result of anthelmintic activity evaluation of ethanol extract of *gerbera gossypina* (Royle) Beauverd on the eggs of GINS of sheep by egg hatch tests *in-vitro* are shown in Table 5. The ethanol extract exhibited ovicidal activity against eggs of GINS. The ethanol extract concentration of 50 mg/ml showed significant at 1% level of significant, inhibition rate comparison with negative control whereas compared to ABZ, 50 mg/ml concentration of ethanol extract inhibit egg hatch significantly ($p<0.001$). ABZ at concentration of 25 mg/ml demonstrated greater ovicidal ($p<0.001$) inhibitory activity ranged from 87.1%. The extract concentration showed dose dependent inhibition. The highest inhibition rate of ethanol extract was noted at concentration rate of 50 mg per ml is 82.9%.

Table 5. Mean inhibition percentage of different concentration of *Gerbera gossypina* (Royle) Beauverd ethanol extract on egg hatching of gastrointestinal nematodes of sheep compared with albendazole (ABZ) as positive control and negative control

Treatments	No. of observation	Larva count	Egg inhibition	Percentage of egg hatch inhibition	Level of Significance
Gerbera gossypina (6mg/ml)	5	155.2 (6.22)	45.2 [a] (6.05)	22.4 [a] (3.11)	
Gerbera gossypina (12mg/ml)	5	141.6 (15.04)	58.4[b] (15.04)	29.2 [b] (7.52)	
Gerbera gossypina (25mg/ml)	5	78.0 (9.77)	122[c] (9.77)	61.0[c] (4.88)	*** P<0.001
Gerbera gossypina (50mg/ml)	5	34.2 (5.11)	165.8[d] (5.11)	82.9[d] (2.55)	
ABZ	5	25.8 (8.67)	174.2[d] (8.67)	87.1[d] (4.33)	
NT	5	177.2 (9.73)	22.8[e] (9.73)	11.4[e] (4.86)	
Overall	30	102.0 (60.85)	98.06 (60.79)	49.0 (30.42)	

Note: Figures in parentheses indicate standard deviation and *** indicates significant at 1% level

IV. Discussion

When assessing the qualities of plant extracts, in-vivo tests are more applicable to GINs control techniques in domesticated animals and, hence, highly trustworthy over in-vitro test. Numerous in-vivo studies contrast treated parasitized animals with known concentrations of plant components with untreated controls or commercially available conventional anthelmintic. In this study, the in-vivo results indicated that EPG loads were reduced in 14 days, 21 days, and 28 days by treatment and control groups at 1 % level of significance. The best EPG load reduction was found on 28 days by T3 (250 mg/ml). On day 28 after treatment, T2 with an average FEC reduction 68.10%, and T3 with an average reduction 89.79% was significant ($p<0.01$) compared to NT, while reduction by T2 was insignificant compared to ABZ, while T3 was significant compared to ABZ at 1 % level of significance. With dosing amount of 250 mg/kg BW, the treated group's FEC decrease rate peaked at 89.79% on 28th days after treatment. This study found similar results to Aktaruzzaman et al. (2015), who found efficacy of anthelmintic medicine against GI nematodes was 90.11% on the 28th day. Similarly, Khan et al. (2018) reported 70.27 percent reduction in FEC % of naturally infected animals. The maximum reduction rate of FEC after 14 days of following treatment with 200 mg/kg dose was reported by Al-shaibani et al. (2009).

Alkaloids, flavonoids, glycosides, tannins, saponins, steroids, and triterpenoids (Carvalho, 2007) were also detected as active ingredient. On day 21 after medication, an efficacy that is almost equal to this observation is achieved with a dosage of 200 mg/kg. Bachaya et al. (2009), observed a maximal FECR% of 87.3% with a crude methanol extract of T. arjuna at 3 g/kg. Another comparable finding was published by Iqbal et al. (2005), who found ECR % was 88.4 and 77.8 @ 3 g per kg BW in sheep on 1st week and after 10 days following treatment, respectively, and concluded that Calotropis procera plant have better anthelmintic properties towards helminthes because they contain bioactive compounds such as alkaloids, carbohydrates, glycosides (Sharma & Sharma, 1999). The findings of this study reported a close efficacy with the above finding of egg count percent reduction (FECR). The greatest decrease (50.5%) in faecal egg count for A. donax was reported by Badar et al. (2022) at 7 g kg-1, which is lower than in this finding. The study demonstrates dose-dependent anti-parasitic effectiveness because tannins encourage physiological responses in the recipient's stomach and trigger the secretion of mucus plus toxic substances here to parasite or combinations of tannin, protein, and amino acids may inhibit egg hatch, resulting in decrease of number of parasitic loads (Kahn & Diaz-hernandez, 1999).

In accordance with the recommendations of WAAVP, a decrease in fecal egg counts of ninety-nine percent or more are regarded as highly efficient, while a decrease of eighty percent is regarded as sufficient (Githiori et al., 2006). At dosages of 250 mg/ml evaluated in sheep naturally infected with Gl nematodes, crude ethanol extracts of *Gerbera gossypina* (Royle) Beauverd reduced faecal egg counts by more than 89.79%. In light of these recommendations, it may be hypothesized that *Gerbera gossypina* (Royle) Beauverd has sufficient anthelmintic activity and might serve as a viable substitute for traditional anthelmintic in small animals.

In-vitro assays and studies to screen anti-parasite effects of plant extracts are low-cost and quick-turn around, allowing for large-scale plant screening. These tests evaluated the influence of anthelmintic efficacy on parasite growth, development, and motility on the drug's pharmacologic and pharmacokinetic qualities without affecting with the host's internal physiological functions (Githiori et al., 2006). In this study, the anthelmintic efficacy of ethanolic extract of *Gerbera gossypina* (Royle) Beauverd was evaluated by egg hatch in-vitro. The frequency of egg hatching observed in the absence of ethanol extract of *Gerbera gossypina* (Royle) Beauverd showed that pure water did not obstruct the eggs' normal development. Furthermore, in the present study ethanol extract exhibited ovicidal activity on the egg hatching of GINS with the best effectiveness at a dose of 50 mg/ml

exhibiting a considerable inhibitory activity against the negative control, but ethanol extract exhibit egg hatch significantly ($p<0.001$) compared to ABZ. The maximum ovicidal reduction rate was seen with ABZ (25 mg per ml), ranging from 87.1% to 100%. At concentrations larger than 50 mg/mL, a crude methanol extract of the P. granatum Linn. reported a good impact, and extracts were more beneficial, as per Jabeen et al. (2015), research.

Our results are in agreement with this analysis. In a recent review, Ahmed et al. (2020), found egg-hatching inhibition with 98.67% and that all of the extracts under study contained alkaloids, saponins, flavonoids, tannins, glycosides, and phenols. The results were similar to those of this study (82.9%). Another similar finding was obtained by Olayemi et al. (2019); Maphosa et al. (2010), and Eguale et al. (2007), who reported the maximum concentration for the aqueous extract to elicit complete (100%) egg hatch inhibition was taken from seeds of Coriandrum sativum and suggested the chemical components' sources, which are thought to be the basis for many medicinal plants' extensive therapeutic effects plants. Eguale et al. (2007b), demonstrated completely inhibited egg hatching by ripe fruits of H. helix with 2 mg/ml dose rate. Suggested might be because the fruits of H. helix include alkaloids, polyphenols, phytosteroides, saponins, and flavonoids, which are the chemical components that give numerous medicinal plants their wide range of therapeutic effects. Tresia et al. (2016), stated that the active components reported in plants were associated to the cuticle damage and changes in the permeability of the larvae cuticle. When the cuticle of larvae is damaged, the protective role and nutrient absorption ability are interfered, that leads to malnutrition and reduced motility, which ultimately causes larval death. This might be due to the active components reported in plants.

Numerous research had conducted on using several types of plants as effective substitutes to traditional anthelmintic in managing parasitic infestations in sheep (Githiori et al., 2006). This study's findings will assist in the development of herbal anthelmintic that are more sustainable, effective, and safe than currently available synthetic anthelmintic.

Therefore, quality-controlled extracts of the complete plants of *Gerbera gossypina* (Royle) Beauverd or perhaps selected bioactive components could be attractive future substitutes for conventional anthelmintic for treating small ruminant GI nematodes. Such a treatment could be employed to cure intestinal nematodes in both traditional and natural agricultural system. It is essential to conduct additional study into the standardization of dose rate, toxicity, and knowing the mechanism or method of action, as well as thorough pharmacokinetics.

V. Conclusion

The whole plant of *Gerbera gossypina* (Royle) Beauverd was found to be effective as an anthelmintic when administered at a dose amount of 250 mg/kg. The maximum ovicidal activity (82.7%) was seen in in-vitro tests using ethanolic extracts at a level of 50 mg/ml. From this study, *Gerbera gossypina* (Royle) Beauverd was found as the most promising plant, utilized to reduce nematodes in sheep. *Gerbera gossypina* (Royle) Beauverd could be promoted as ethno veterinary plant to control nematode infestations in sheep of Nepal. These studies help to validate herbs and provide scientific support for commercial production and protection of such plants. Additional research on artificially induced parasite infections, bioactive components, repeatability, dose, administration regimen, toxic effects, and efficacy of *Gerbera gossypina* (Royle) Beauverd may be required. Furthermore, based on genetic or environment variations, plant developmental status during harvest, drying processes, and storage methods, chemical components might vary significantly among individual plants.

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