



CORRELATION BETWEEN THE PHYTOCHEMICAL CONSTITUENTS AND ANTHELMINTIC ACTIVITY OF *LAWSONIA INERMIS* LEAF EXTRACTS

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ABSTRACT

To know the anthelmintic activity of leaves of *Lawsonia inermis*, we used maceration method of extraction using solvents like ethanol and methanol. Each extract was tested for anthelmintic activity by following standard method. The ethanolic and methanolic extract displayed anthelmintic activity in dose dependant manner. We found that the ethanolic as well as methanolic extracts were more potent than the positive control as far as their anthelmintic activity was concerned. To correlate phytochemical screening with anthelmintic activity, phytochemical evaluation of the extract was also performed. From our result it may be mentioned that the ethanolic extract was potent as anthelmintic agent due to the presence of glycosides, terpenoids and flavonoids. On the other hand, the methanolic extract was effective probably due to the involvement of alkaloids, glycosides, terpenoids and flavonoids.

KEYWORDS- *Lawsonia inermis*, ethanolic extract, methanolic extract, phytochemical screening, anthelmintic activity.

INTRODUCTION

Plant materials have been used for the treatment of serious diseases throughout the world before the advent of modern clinical drugs¹. The use of medicinal plants still plays an important role to cover the basic health needs in the developing countries.² Several top selling drugs of modern times such as Quinine, Artemisinin, Shikonin, etc. are obtained from plants¹. Most of the phytochemicals, secondary metabolites of plants, are physiologically active². The plants are known to provide a rich source of botanical, anthelmintic, antibacterials, and insecticides.³ Helminths are recognized as a major problem to livestock production throughout tropics. Most diseases caused by helminths are of a chronic and debilitating in nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. The parasitic gastroenteritis is caused by mixed infection with several species of stomach and intestinal worms, which results in weakness, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity. Most of the existing anthelmintics produce side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhoea. Chemotherapy is the only treatment and effective tool to cure and control helminth infection, as effective vaccines against them have not been developed so far. Indiscriminate use of synthetic anthelmintics can lead to resistance of parasites. Herbal drugs have been in use since ancient times for the treatment of parasitic disease in human and could be of value in preventing the development of resistance⁴. Some investigators have mentioned the importance of some phytochemicals like alkaloids, glycosides, terpenoids, tannins and flavonoids for showing anthelmintic activity of plants^{3,5,6}.

Natural drugs are obtained from the plant, animal or mineral kingdom. The plant kingdom is the store house of the organic compounds⁷. *Lawsonia inermis* Linn (Family-

Lythraceae) is commonly known as henna and mehendi. It is a small shrub to a height of 6 m. The branches of this plant are lateral with leaves that grow in pair which are 2-4cm long. The flowers are fragrant and red rose like⁸. Henna leaves contain an important pigment called "lawsone". It contains tannic acid, gallic acid, mucilage and naphthaquinone. It is also known as good medicinal plant which is said to have properties of astringent, antihemorrhagic, intestinal, neoplastic, hypotensive and sedative effects⁹. Useful parts of the plant are leaf, flower, bark, root and seed. The leaf used for alleviating jaundice, skin disease, venereal disease and small pox. Seeds are effective against dysentery, liver disorders and associated problems. Bark is used for burn scald. Root is considered as a potent medicine for gonorrhoea and herpes¹⁰. Some workers have mentioned that the *in vitro* anthelmintic potency of the petroleum ether extract (obtained by maceration method) of *Lawsonia inermis* leaves using Indian earthworm (*Phretima posthuma*). Considering it as a potential anthelmintic agent, we undertook a study to know the anthelmintic property of the methanolic and ethanolic extracts of *Lawsonia inermis* leaves¹¹.

MATERIALS AND METHODS

Plant Material

The leaves of the plant *Lawsonia inermis* were collected from Chhend, Rourkela, during December 2011. The sample was authenticated by Dr. Pratiba Sahoo, Botanist, Rourkela Autonomous College, Rourkela. The shade dried leaves were powdered and stored in a dessicator.

Preparation of Extracts

The powdered leaves were passed through a sieve (No.40) and stored in a dessicator. Then the powder (10gm) of *Lawsonia inermis* were extracted by using maceration method. The powdered leaves were macerated in 60ml of ethanol for 3 days at room temperature. The resulting extract was filtered through a filter paper (Whatman

No.1). The residue was further extracted using the same procedure. The filtrates obtained were combined and then evaporated to dryness under reduced pressure. Instead of using ethanol, the above mentioned procedure was conducted separately for methanol¹².

Phytochemical screening¹³

Following chemical tests were performed for testing different chemical groups present in both the extracts:

Alkaloids

Mayer’s test:-To 2-3 ml of the extract, few drops of the Mayer’s reagent (1.36gm of Mercuric chloride and 5gm of Potassium iodide in 100ml distilled water) were added. Formation of a cream colour precipitate indicated the presence of alkaloids.

Amino acids

Millon’s test:-To 2 ml of the test extract about 2ml of Millon’s reagent (Mercury nitrate) was added. White precipitate indicated the presence of amino acids.

Carbohydrates

Molish test:-To 2 ml of the test extract, at first, few drops of alcoholic α -naphthol were added. Then through sides of test tube, few drops of concentrated sulphuric acid were mixed with it. Purple to violet colour ring appeared at the junction indicated the presence of carbohydrates.

Flavonoids

Alkaline reagent test:-To 2 ml of the test extract, few drops of sodium hydroxide solution were added. At first, intense yellow colour was formed, which was subsequently turned to colourless, on addition of few drops of dilute acid indicated the presence of flavonoids.

Glycosides

Borntrager’s test: The test extract was boiled with 1 ml of sulphuric acid in a test tube for 5minutes. While hot it was filtered, then it was cooled. Shaking of the mixture was done with equal volume of chloroform. Two layers of solution were formed. The lower layer of chloroform was separated. Then that layer was shaken with half of its volume of dilute ammonia. Production of a rose pink to red colour suggested the presence of glycosides.

Saponins

Froth formation test :- 2 ml of the extract was shaken vigorously with water in a test tube. Formation of persistent foam indicated the presence of saponins.

Tannins

Gelatin test :- To 2 ml of the extract, 1% gelatin solution containing 10% sodium chloride was added. Formation of a precipitate suggested the presence of tannins.

Proteins

Warming test :- 2 ml of the extract was heated in a boiling water bath. Proteins get coagulated due to heating.

Steroids and Triterpenoids

Salkowski test :- The test extract was treated with few drops of concentrated sulphuric acid. Red colour at lower layer indicated the presence of steroids, whereas formation of yellow colour at the lower layer suggested the presence of triterpenoids.

Anthelmintic Activity

The anthelmintic activity was performed on adult Indian earthworm *Pheritima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings¹⁴. Indian adult earthworms, collected from moist soil and washed with normal saline to remove all fecal matter, were used for anthelmintic activity. Different concentrations of the dried extracts (10-25mg/ml in distilled water with tween 80) were prepared¹⁵. 10ml of each concentration of ethanolic extract was delivered into a Petridish .Then six worms (same type) were placed in it. Similarly, for each concentration of methanolic extract, 6 worms were used. Time for paralysis was noted when the worm did not revive even in normal saline. Time for death of worms were also recorded when the worms lost their motility followed by fading away of their body colour (when dipped in warm water of 50⁰C). Piperazine citrate (15mg/ml in distilled water) was used as positive control¹⁶.

Statistical analysis

Data were analysed using one way factorial ANOVA tests followed by Dunnett’s t-tests on each group. P values under 0.001 were considered highly significant¹⁷.

RESULTS

While amino acids, flavonoids, glycosides, saponins, proteins and triterpenoids were found in the ethanolic extract, alkaloids, flavonoids, glycosides, saponins, proteins and triterpenoids were available in the methanolic extract. In the ethanolic extract, alkaloids, carbohydrates, steroids and tannins were absent. On the other hand, amino acids, carbohydrates steroids and tannins were not found in the methanolic extract (Table 1).

Considering the time for paralysis and death of the worms, it was found that both methanolic and ethanolic extracts were more or less equally potent. The anthelmintic activity revealed the concentration dependant nature of the extracts. We found that ethanolic as well as methanolic extracts were better than the positive control (piperazine citrate 15mg/ml) as far as their anthelmintic activity was concerned (Figure 1). Negative control (distilled water with Tween 80) did not show any activity against earthworms (Table 2).

Table 1: Qualitative analysis of various leaf extracts of *Lawsonia inermis*

Phytoconstituents	Ethanolic extract	Methanolic extract
Alkaloids	-	+
Aminoacids	+	-
Carbohydrates	-	-
Flavonoids	+	+
Glycosides	+	+
Saponins	+	+
Proteins	+	+
Steroids	-	-
Tannins	-	-
Triterpenoids	+	+

‘+’ = Present, ‘-’ = Absent

Table 2: Anthelmintic activity of the leaf extracts of *Lawsonia inermis* and controls

Treatment	Group	Concentration (mg/ml)	Time of paralysis (min)±SEM	Time of death (min)±SEM
Distilled water with Tween 80 control	I	-	-	-
Piperazine citrate	II	15	97±0.57	116±1.15
Ethanolic extract	III	25	57±0.57***	63±1.15***
	IV	50	35±1.15***	40±1.15***
Methanolic extract	V	25	56±1.52***	65±0.57***
	VI	50	35±1.15***	42±1.52***

Values are expressed as mean±standard error mean (SEM). Values are calculated by using ONE way ANOVA followed by Dunnett's t-test. ***Values are highly significant i.e., more potent than controls at (p<0.001).

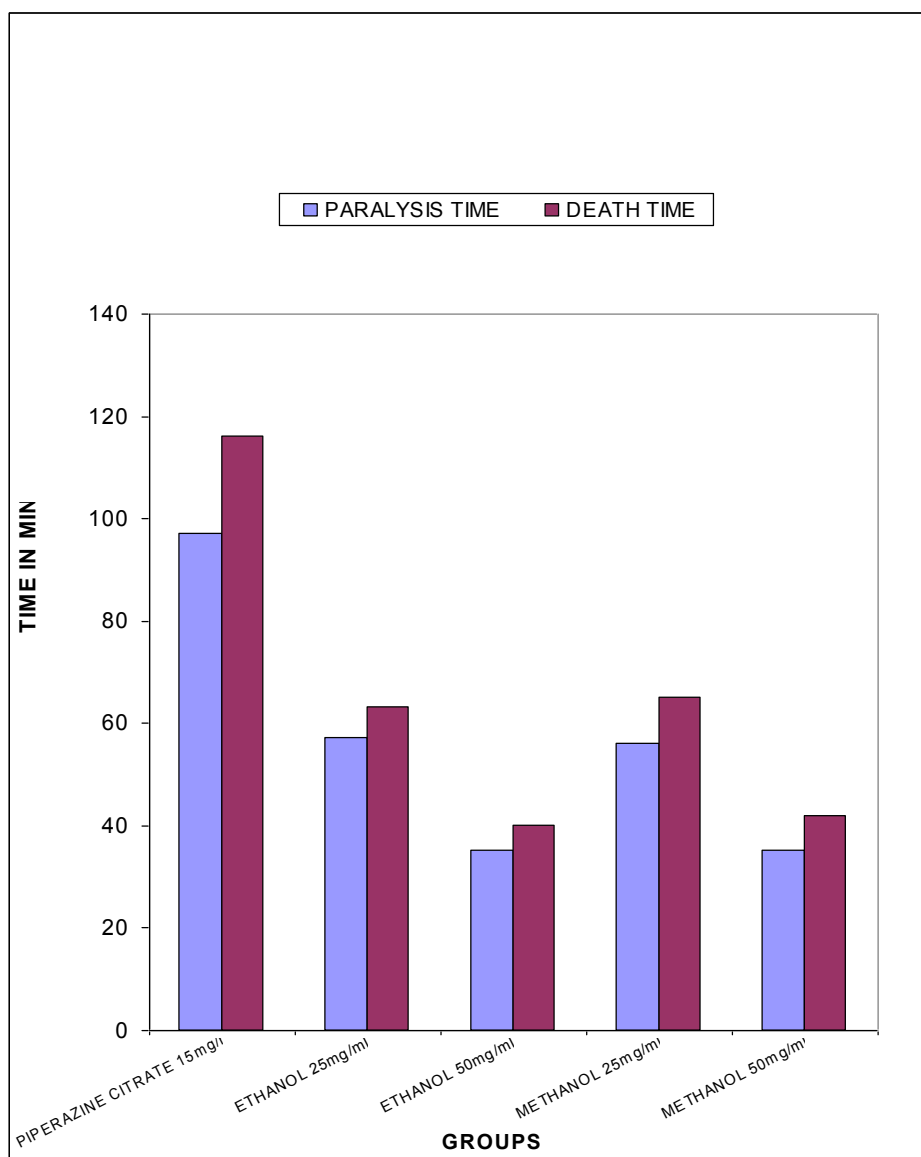


Figure 1: Anthelmintic Activity of *Lawsonia inermis* leaf extracts

DISCUSSION

While some investigators have mentioned the importance of some phytochemicals like alkaloids, glycosides, terpenoids, tannins and flavonoids for showing anthelmintic activity of plants, we have seen the presence of flavonoids, glycosides, saponins, proteins, triterpenoids, amino acids and alkaloids in the extracts used in this study^{3,5,6} (Table 1). It seems that glycosides, terpenoids and flavonoids were responsible phytochemical constituents for demonstrating anthelmintic activity of the ethanolic extract. On the other

hand, the anthelmintic activity of the methanolic extract was probably due to the presence of alkaloids, glycosides, terpenoids and flavonoids.

Although Bairagi *et al.* (2011) reported that petroleum ether extract of *Lawsonia inermis* leaves displayed anthelmintic activity, we have seen both methanolic and ethanolic extracts were having potent anthelmintic activity¹¹. Moreover, those extracts were more effective (highly significant) than the positive control as per as their anthelmintic activity was concerned (Table 2).

CONCLUSION

It can be concluded that the phytochemical constituents responsible for anthelmintic activity are present in the ethanolic and methanolic extracts of leaves of *Lawsonia inermis*. To identify the actual phytochemical constituents that are present in the crude drug extracts of this plant which are responsible for anthelmintic activity, should be studied thoroughly. It would be even better to conduct further research on pure chemical constituents of the plant to critically evaluate their activity on many animals.

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