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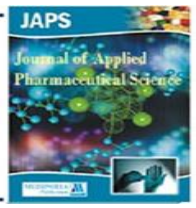

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Anthelmintic activity of aqueous and ethanolic extract of Trikatu Churna

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ABSTRACT

The present study was done with the aim to evaluate anthelmintic activity of Trikatu churna containing traditionally user herbs viz., *Piper nigrum* L. (Piperaceae), *Piper longum* L. (Piperaceae) and rhizome of *Zingiber officinale* Roscoe using adult earthworm Pheritima posthuma. All these three ingredients are spicy, commonly used in our daily diet, also well known for their tremendous therapeutic potential, since from the Vedic period. The aqueous and ethanolic extract of Trikatu churna and its ingredients were also screened for preliminary phytochemical studies. Piperazine citrate was used as standard and it was found that the TCEE activity is higher than TCAE.

Key words: Trikatu Churna, Piperazine citrate, *Pheritima posthuma*, Trikatu Churna ethanolic extract (TCEE), Trikatu Churna alcoholic extract (TCAE)

INTRODUCTION

Helminthosis plays a crucial role in the small ruminant production leading to enormous economic losses particularly in areas where extensive grazing is practiced (Waller, 1997). It is also being recognized as cause of many acute as well as chronic ill healths among the various human beings as well as cattle's. Development of resistance to most of the commercially available anthelmintics became a severe problem worldwide. Moreover, these drugs are unaffordable, inaccessible or inadequately available to the resource-poor farmers of the developing countries (Fajmi and Taiewo, 2005). These factors paved the way for the herbal formulation as alternative anthelmintics. In the current study, we have attempted to investigate the traditional polyherbal formulation for their anthelmintic activity.

Trikatu churna is one of the traditional polyherbal preparations, made up of combination of three important spicy materials, such as *Piper nigrum* L (Piperaceae), *Piper longum* L. (Piperaceae) and *Zingiber officinale* Roscoe. (Zingiberaceae). All these plant materials are used world wide as spices. They are also used as important ingredients in folklore medicine in many Asian countries. However, the consumption of these spices would exert several health beneficial effects by the virtue of their innumerable therapeutic potentials, such as fever, asthma, cold, cough and other general health disorders (Chopra,1992; George, 1972; Namjoshi, 1976). There are number of researches carried out in this particular stream. But so far no clinical trials are made on this compound polyherbal formulation, Trikatu churna. Hence, the present study was undertaken to explore the anthelmintic activity of Trikatu churna. The present study is also aimed to establish its clinical validity.

MATERIALS AND METHODS

Collection of Plant materials

The plant materials of *Piper nigrum* L, *Piper longum* L. along with the fruits were collected from the medicinal garden, Madhagadipet, Puducherry, rhizomes of *Zingiber officinale* Roscoe were collected from the local market of Puducherry. The fruits and the rhizomes of respective plants after collection were shade dried, powdered (40 mesh size) to get a coarse powder.

Preparation of the Trikatu churna

The dried powder material of *Piper nigrum* L (50gm), *Piper longum* (50gm) *and* rhizomes of *Zingiber officinale* Roscoe (50mg) were thoroughly mixed and then sieved through 20 mesh size to get a fine powdered formulation. The prepared churna is stored in airtight container for further processing.

Preparation of the aqueous extract

The 100g of Trikatu churna and its ingredients were extracted in water at 50 - 60°C in a soxhlet apparatus separately. The extract was concentrated to dryness in a water bath at controlled temperature (50 -60°C). The dried 95% of the aqueous extract weighed in a required dose and dissolved in known volume of distilled water, separately for further treatment.

Preparation of ethanolic extract

The 100g of Trikatu churna and its ingredients were extracted in 95% ethanol at 50 - 60°C in a soxhlet apparatus separately. The extract was concentrated to dryness in a water bath at controlled temperature (50 -60°C). The dried 95% of the ethanolic extracts weighed in a required dose and dissolved in known volume of distilled water, separately for further treatment.

Preparation of Standard Solution

Piperazine citrate is taken as standard drug and the concentration of the standard drug was dissolved in 100ml of normal saline solution to get 1, 2, and 4ml of solution. Normal saline alone was used as control.

Phytochemical Evaluation of the Crude Extracts

Phytochemical screening of the extracts for the presence of secondary metabolites were performed using the following reagents and chemicals: for alkaloids with Mayer's, Wagner's and Dragendroff's reagents, for flavonoids with the use of Mg and HCl, tannins with 1% gelatin and 10% NaCl solutions, for saponins with distilled water (Harborne, 1998; Namjoshi, 1976; Nooman et al., 2008; Nawagish et al.,2007).

Evaluation of anthelmintic activity

Adult earth worms (*Pheretima posthuma*) were collected (due to their anatomical and physiological resemblance with the intestinal round worm parasites of human beings) Earth worms was thoroughly washed with normal saline to remove the adhering material. Petridishes of equal size were collected and 20ml of normal saline alone was poured in the first petridish, 20ml of Piperazine citrate solution of concentration 1, 2 and 4mg/ml were poured in second, third and fourth petridishes, respectively. Then 20ml (4mg/ml) of the test solutions that is, the aqueous and ethanolic extracts of Trikatu churna were taken in fifth, sixth petridishes respectively. Placed six earthworms of nearly equal size in each petridish and time taken for the induction of paralysis (motion less) and complete death of earthworms was noted. The experiment was repeated thrice and confirmed the readings (Dwivedi et al., 2008).

Statistical Analysis

All the data are expressed as mean ± S.E.M. (standard error of the mean). The significance level was determined using the Student's't' test. A p value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The results of the above studies demonstrated that, the ethanolic extract of Trikatu churna shows potent anthelmintic activity with varying magnitudes. But the extract of Trikatu churna showed highest activity, which is almost equal in effectiveness to standard Piperazine citrate. The difference in the time taken for induction of paralysis in both Piperazine citrate and Trikatu churna was insignificant or almost same. However, significant difference was observed when compared the induction of paralysis time of Piperazine with aqueous extracts. The mode of action for the piperazine is generally by paralyzing parasites, which allows the host body to easily remove or expel the invading organism (Table 1). The preliminary phytochemical observations of the ethanolic extracts of Trikatu churna and its plant ingredients have shown the occurrence of alkaloids, flavonoids, tannins, lignins and steroids (Table 2). It indicates that, the Trikatu churna is a mixture of all these phytoconstituents and interaction of all these chemicals might be resulted in synergistically enhanced therapeutic efficacy of anthelmintic activity.

Table 1: Anthelmintic activity of Aqueous and ethanolic (95%) extracts of Trikatu churna and Piperazine citrate.

Sl. No	Treatment	Concentration (mg/ml)	Paralysis Time (min)	Death time (min)
01	Normal Saline	0.9% NaCl	No paralysis	No death
02	Piperazine Citrate	01	043.66 ± 1.071	063.33 ± 0.838
03	Piperazine Citrate	02	030.00 ± 0.881	066.66 ± 1.071
04	Piperazine Citrate	04	021.33 ± 0.509	036.33 ± 1.895
05	*TCAE*	04	046.00 ± 0.881	235.33 ± 1.503
06	TCEE	04	029.66 ± 0.693	090.66 ± 1.347

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Table 2: Distribution of primary and secondary metabolites in Trikatu churna and its ingredients.

Tests		*Piper nigrum*	*Piper longum*	*Zingiber officials*	Trikatu churna
Alkaloids	Mayer's test	+	+	+	+
	Wagner's test	+	+	+	+
	Dragendroff's test	+	+	+	+
Steroids	Salkowski' test	-	+	+	+
	Libermann and Burchard test	-	+	-	+
Flavonoids	Extract + Mg turnings	+	+	+	+
	Extract + Aqueous	+	+	+	+
	NaOH + Conc H_2SO_4	+	+	+	+
Saponins	Foam test	+	-	+	+
Tannins	Gelatin test	+	-	+	+
Lignans	Lignan test	+	+	+	+

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