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Anthelmintic Activity of Trikatu Churna and its Ingredients

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Abstract

The alcoholic extract of Trikatu churna and its ingredients were evaluated for anthelmintic activity. The dried fruits of *Piper nigrum* L. (Piperaceae), *Piper longum* L. (Piperaceae) and rhizome of *Zingiber officinale* Roscoe. (Zingiberaceae) were powdered and mixed together in equiproportions to get a polyherbal formulation, Trikatu churna. 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 alcoholic extract of Trikatu churna and its ingredients were screened for preliminary phytochemical studies and also tested for anthelmintic activity against *Pheritima posthuma* and recorded the time taken for induction of paralysis and death. Piperazine citrate (10 mg/ml) was included as standard reference and distilled water as control**.** The results demonstrated that, the extracts of Trikatu churna and its plant ingredients showed the presence of alkaloids, flavonoids, tannins, lignins and steroids, these test samples were also exhibited potent anthelmintic activity, but the highest activity was noticed in Trikatu churna, this might be due to the multifunctional effect of all the three plant ingredients of Trikatu churna. Based on the above results, it is confirmed that, combination of *Piper nigrum*, *Piper longum* and *Zingiber officinale* in Trikatu churna offered promising anthelmintic effect than using the ingredients alone.

Keywords: Trikatu Churna, Piperazine citrate, *Pheritima posthuma.*

Introduction

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, asthama, cold, cough and other general health disorders (Anonymous, 1985; Chopra, *et al*, 1992; George watt, 1972; Rakesh and Sushil, 2003; Namjoshi, 1976; Sivarajan and Indira Balachandran, 1996)

There are voluminous research carriedout in this particular stream, a brief review of the notable work is highlighted, such as Krimikuthar Ras, Sanjivani vati, Kumari asava, Bhallakasava, Vidanasava and the combination of two or more these formulations were tested for anthelmintic activity against *Pheritima posthuma*. (Nirmal *et al,* 2008), aqueous extract of fruits of *Terminalia chebula* Retz. (Dwivedi *et al,* 2008). 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 and compared its effects to its individual ingredients. The present study is also aimed to establish its clinical validity.

Materials and Methods

The anthelmintic activity was tested on earth worms (*Pheretima posthuma)* using ethanolic (95%) extracts of *Piper longum, Piper nigrum, Zingiber officinale* and Trikatu churna. The crude extracts of Trikatu churna and its ingredients were also screened for preliminary phytochemical studies to find out the occurrence of possible major chemical groups in the given extracts.

Collection of Plant materials

The plant materials of *Piper nigrum* L, *Piper longum* L. along with the fruits were collected from the Agricultural University, Bangalore, rhizomes of *Zingiber officinale* Roscoe. were collected from the farmers of Gulbarga in the month of October-November. All these plants were authenticated and the voucher specimens were deposited as Herbarium in Department of Botany, Gulbarga University, Gulbarga (Karnataka, India). The fruits and the rhizomes of respective plants were surface sterilized by using 50% alcohol, then shade dried and powdered for the preparation of Trikatu churna.

Preparation of the Trikatu churna

The Trikatu churna is a fine powder of drugs. It is prepared by mixing equal quantities of the powder of the dried fruits of *Piper nigrum, Piper longum* and rhizomes of *Zingiber officinale* and then sieved through muslin cloth. This churna is stored in airtight container for further processing (Rakesh and Sushil, 2003).

Preparation of the 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 flash evaporator (Buchi type) under reduced pressure and 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.

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; Sadasivam and Manickam, 1992; Ogbonnia, *et al*, 2008; Nooman, *et al*, 2008; Mohd. Nawagish *et al*, 2007).

Preparation of Standard Solution and Control

Piperazine citrate powder standard drug [Adani Pharmachem Pvt. Ltd, Gujarat, India] was dissolved in 100ml of normal saline solution to get 1, 2, and 4ml of solution. Normal saline alone was used as control.

Experiment Design

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 were 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 ethanolic extracts of *Piper nigrum*, *Piper longum*, *Zingiber officinale* and Trikatu churna were taken in fifth, sixth, seventh and eighth petridishes, respectively. Placed six earth worms of nearly equal size in each petridish and time taken for the induction of paralysis (motion less) and complete death of earth worms 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 alcoholic extract of Trikatu churna and its individual ingredients possess 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 ingredients of churna alone. The mode of action for the piperazine is generally by paralysing parasites, which allows the host body to easily remove or expel the invading organism (Table 1). The preliminary phytochemical observations of the alcoholic extracts of four different test samples such as 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 all these phytoconstituents and interaction all these chemicals might be resulted in synergistically enhanced therapeutic efficacy of anthelmintic activity.

Table 1. Anthelmintic activity of ethanolic (95%) extracts of *Piper longum, P.nigrum, Zingiber officinalis,* 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	*Piper longum* Linn	04	113.66 ± 1.347	207.00 ± 1.201
06	*Piper nigrum* Linn	04	067.66 ± 0.769	238.66 ± 4.717
07	*Zingiber officinale*	04	046.00 ± 0.881	235.33 ± 1.503
08	Trikatu churna	04	029.66 ± 0.693	090.66 ± 1.347

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

Tests		*Piper nigrum*	*Piper longum*	*Zingiber officinalis*	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	Labat test	-	+	-	+
	Lignan test	+	+	+	+

The main finding from this investigation is that the alcoholic extracts from all four test samples produced paralysis of earth worms after 30-120 minutes when treated 4mg/ml concentration of the Trikatu churna and individual extracts. Moreover earthworms did not get recovery from paralysis even after 2-3 hours of post treatment period. Chemotherapeutic drugs against helminthes infection act mainly through three different mechanisms, such as, disruption of the neuromuscular physiology, blocking the energy metabolism, disrupting the highly efficient reproductive system of the parasites (Geary *et al*, 1992). Several important anthelmintics cause paralysis by disrupting one or the other aspect of neuromuscular system (Loukas and Hotez, 2005). The paralytic effect of alcoholic extract of *Piper longum* on *Gigantocotyle explanatum* by progressive reduction in the spontaneous muscular activity, which may be associated with their inhibitory effect on the neuromuscular system of the amphistome (Singh *et al,* 2008). The anthelmintic activity of *Zingiber officinale* and *Piper longum* a chief ingredients in Krimikuthar Ras, Sanjivani vati and Vidangasava, an Ayurvedic formulations were reported as potent anthelmintic agents (Nirmal *et al*, 2008). In vivo anthelmintic activity of ginger was confirmed against gastrointestinal nematodes of sheep, thus justifying the age-old traditional use of this plant in helminth infestation (Iqbal *et al*, 2006). The oil of *Piper longum* elicited the pronounced effect on the rhythmic movements of *Ascaris* as evidenced from resulting paralysis that occurred between 12 to 15 minutes of exposure with 1:1000 v/v concentration of oil. The essential oil of *Piper longum* and its non-phenolic fraction did not significantly differ in their ability to inhibit the rhythmic motions, even though; minimal motions persisted in case of non-phenolic fraction (Kokate *et al*, 1980). Thus, it has happened obviously that, Trikatu churna is a mixed preparation of all these useful phytoconstituents, perhaps the synergistic interaction of alkaloids, flavonoids, tannins, lignins, steroids and other constituents in the extract may impart strong anthelmintic activity to the poly herbal preparation. But the mechanism involved in the interaction between the different plant extracts remain

unclear and should be further evaluated.

Conclusion

Trikatu churna was found to possess higher the rate of phytoconstituents and promising anthelmintic activity. It is also confirmed that, these spicy products triggers natural immune system to fight against various parasites as well as helminthes. This study would provide the preliminary scientific evidence for the folkloric, ethno-botanical and traditional use of this churna for destruction of helminthes / parasites and eliminates from host body and other health benefits.

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