ANTIMICROBIAL AND ANALGESIC ACTIVITIES OF TRIKATU CHURNA AND ITS INGREDIENTS

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Summary

Trikatu churna is one of the Ayurvedic herbal preparations, practice against certain general health disorders such as cold, cough, appetizer, carminative, purgative etc. In the present study phytochemical, antibacterial, antifungal and analgesic activities of ethanolic extracts of Trikatu churna (equiproportions of powdered fruits of *Piper nigrum* L, *Piper longum* L and rhizome *Zingiber officinale* Roscoe.) and its individual components were tested for antimicrobial activity against certain clinical bacterial and fungal isolates, such as *Escherichia coli, Staphylococcus aureus, Aspergillus niger* and *Mucor* species by *in-vitro* agar well diffusion method and analgesic activity by *in-vivo* hot plate method using mice. Trikatu churna was found to possess higher the rate of phytoconstituents, promising antimicrobial (non-specific) and moderate analgesic activities.

Key Words: Trikatu churna; Agar well diffusion technique; Hot plate method; Antibacterial; Antifungal; Analgesic activity

Introduction

Trikatu churna is one of the traditional polyherbal preparation, formed by the mixture of three important spicy materials, such as *Piper nigrum* L. (Piperaceae), *Piper longum* L. (Piperaceae) and *Zingiber officinale* Roscoe. (Zingiberaceae) (1). All these plant materials are extensively used as one of the chief constituents in various Ayurvedic compound preparations (2) and healthcare preparations (3). The antioxidant, antitumor, anticancer and other pharmacological effects of *P. nigrum, P. longum* and *Z. officinale* are mainly due to pungent constituents such as 6-gingerol, piperine, capsaicin, volatile oil and other bioactive molecules (4-8)

Few research studies have been carriedout on these individual ingredients, but so far no clinical trials have been made on this compound polyherbal formulation, Trikatu churna. Hence, the present study was undertaken for the systematic evaluation of the antimicrobial and analgesic activities of this polyherbal formulation, Trikatu churna and compared its effect to its individual ingredients.

Materials and Methods

The present study was undertaken to assess the antibacterial and antifungal activities of different concentrations of ethanolic extracts of Trikatu churna and its plant ingredients on highly pathogenic bacteria like *Escherichia coli, Staphylococcus aureus* few pathogenic fungi such as *Aspergillus niger* and *Mucor* species. Analgesic activity was studied the maximum pain tolerance of Trikatu churna in pain induced mice.

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 are deposited in Herbarium of Department of Botany, Gulbarga University, and Gulbarga. 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 Trikatu churna: The pre-cleaned dried powdered fruits of *Piper longum*, *P.nigrum* and rhizomes of *Zingiber officinale* were taken in equal proportions and mixed well. This churna was stored in an airtight container for further processing

Preparation of the Extracts: The 100g of Trikatu churna and its plant ingredients were extracted with 90% alcohol at 50 - 60°C in a soxhlet apparatus. The different extracts were collected in a separate container and concentrated to dryness in a flash evaporator (Buchi type) under reduced pressure and controlled temperature (40 - 50°C) and note down the yield of crude extracts.

Microorganisms Used: Clinical laboratory bacterial isolates of *Staphylococcus aureus*, and *Escherichia coli* and pure fungal isolates like *Aspergillus niger* and *Mucor* species obtained from the Department of Microbiology, Gulbarga University, Gulbarga.

Culture Media: The nutrient agar (antibacterial activity) and Sabouraud's dextrose agar (antifungal activity) were purchased from HiMedia, Laboratories Limited, Mumbai. Nystatin an antifungal agent, purchased from, Tocelo chemicals, Netherlands, Streptomycin sulphate another antibiotic from Nanjing Asian chemicals Co., Ltd., Analgin by Vani Pharma labs Limited, Hyderabad.

The Preliminary Phytochemical Studies: The alcoholic extracts of Trikatu churna and its plant ingredients were tested by applying general chemical tests for alkaloids, tannins, steroids, terpenoids, phenols, flavonoids, saponins, etc (9-13).

Antimicrobial activity: Here the *in-vitro* antibacterial and antifungal activities were assayed by using agar well diffusion method. The pure cultures of different pathogens were grown overnight in sterile nutrient broth and incubated at 37°C for 24 hours, then subjected for optical density of the overnight incubated culture were adjusted to 0.1 at λ_{600} with sterile nutrient broth. The 0.1ml of the culture was seeded on 25 ml of solidified nutrient agar plate and Sabouraud's dextrose agar plates for bacterial and fungal cultures. The wells were bored with 8mm borer in seeded agar, and then the particular concentrations (500µg/0.2ml) of the extracts of Trikatu churna and its ingredients were added separately in each well. Soon after the plates were then kept at 10°C for 30min. After it normalized to room temperature plates were incubated at 37°C for 24hr. After incubation period was completed, the zone of inhibition was measured and recorded (14).

Analgesic activity: The analgesic activity of the ethanolic extract of Trikatu churna was studied by hot plate method (15). Swiss albino mice weighing between 20 -25g and heat sensitive were selected for experimental work. Then mice were randomly divided into five groups of 6 mice each. The first group served as control and received the vehicle only (ie., 0.2ml of distilled water per animal). The second, third, fourth, fifth and sixth groups of mice have received the alcoholic extract of Trikatu churna suspended in distilled water at 50, 100, 150, 200 and 250mg/kg b.wt., respectively. The seventh group received the standard analgin at a dose of 40mg/kg b.wt. All the treatments were administered intraperitoneally. The observations were made at 0, 30, 60 and 120 minutes.

Statistical analysis: Results are expressed as mean $\pm$ standard error of mean (SEM). The data obtained from the above studies were analyzed using Student's paired't' test. P values less than 0.05 were considered significant.

Results

The results demonstrated that, all the four extracts of *Piper nigrum, Piper longum, Zingiber officinale* and Trikatu churna possess potent antimicrobial and moderate analgesic activities.

Preliminary Phytochemical Screening: Qualitative Phytochemical analyses were carried out with the alcoholic extract of Trikatu churna and its ingredients in order to determine the presence of plant secondary metabolites. The results of the above studies clearly demonstrated the occurrence of alkaloids, phenols, tannins, flavonoids, steroids, lignins and saponins in all four different extracts. But the steroids are absent in *Piper nigrum,* where as saponins and tannins are absent in *Piper longum.* Labat test for lignin is negative to *Zingiber officinale*. However, the Trikatu churna extract has shown positive to all the tests, indicates that, the Trikatu churna is a mixture of all these phytoconstituents (not tabulated).

Antibacterial activity: The clear circular zone of inhibition formed around the well containing the plant extract and the diameter of the inhibition zone was taken as the sensitivity index of the bacteria to plant extract. The data in the Table-1 indicates that, Trikatu churna was proved to be very effective against *Escherichia coli* and *Staphylococcus aureus*, but the moderate activity was noticed with all the individual ingredients of Trikatu churna against *Escherichia coli* and *Staphylococcus aureus.*

Table-1 Antibacterial activity of Trikatu churna and its ingredients.

Sl. No	Treatment	Concentration (μg)	Zone of Inhibition (mm)	
			Escherichia coli	*Staphylococcus aureus*
01	*Piper nigrum* L.	500	12 ± 0.12	13 ± 0.21
02	*Piper longum* L.	500	14 ± 0.13	15 ± 0.32
03	*Zingiber officinale. Roscoe.*	500	13 ± 0.15	15 ± 0.41
04	Trikatu churna	500	17 ± 0.11	16 ± 0.12
05	Streptomycin sulphate	50	20 ± 0.12	19 ± 0.01
06	Control (Sterile Distilled Water	0.1 ml	nil	nil

Antifungal Activity: Inhibition of radial growth of the fungi over a period of 48 hours at 28°C in a medium amended with plant extract is taken as an indication of the antifungal activity of the plant extract as against the control. The data in the table-2 indicates that, the Trikatu churna and its ingredients have antifungal property. But the percentage inhibition of radial growth varies among them. Among, *Piper nigrum* has shown mild activity against both the fungi. Where as *Piper longum* and *Zingiber officinale* have shown significant antifungal activity, but Trikatu churna, a compound drug has shown highest activity against both the fungi tested

Table-2 Antifungal activity of Trikatu churna and its ingredients

Sl. No	Treatment	Concentration (μg)	Zone of Inhibition (mm)	
			Aspergillus niger	*Mucor* species
01	*Piper nigrum* L.	500	4.1 ± 0.10	3.9 ± 0.01
02	*Piper longum* L.	500	5.5 ± 0.14	5.0 ± 0.12
03	*Zingiber officinale. Roscoe.*	500	5.8 ± 0.11	5.5 ± 0.11
04	Trikatu churna	500	6.2 ± 0.01	7.0 ± 0.05
05	Nystatin	50	9.0 ± 0.12	10 ± 0.11
06	Control (Sterile Distilled Water)	0.1 ml	nil	nil

Analgesic activity: The hot plate method is used for evaluating centrally acting analgesic effects of the Trikatu churna at different doses of 50, 100, 150, 200, 250 mg/kg b. wt. was the increase the reaction time was recorded (Table-3). But increase in the pain threshold was noticed only at the dose of 250mg/kg b.wt of the Trikatu churna.

Table-3. The analgesic activity of Trikatu churna on Swiss albino mice using hot plate method

Sl. No	Treatment	Dose (mg/kg b.wt)	Pain Threshold (in seconds)			
			15 min	30 min	45 min	60 min
01	Distilled Water	1.0ml	2.17 ± 0.23	2.45 ± 0.05	2.40 ± 0.34	2.52 ± 0.17
02	Trikatu churna	50	2.45 ± 0.12	2.42 ± 0.09	2.65 ± 0.12	3.35 ± 0.23
03	Trikatu churna	100	3.15 ± 0.12	3.27 ± 0.30	3.07 ± 0.09	3.75 ± 0.15
04	Trikatu churna	150	3.20 ± 0.24	3.27 ± 0.23	3.35 ± 0.41	3.17 ± 0.09
05	Trikatu churna	200	3.17 ± 0.28	3.32 ± 0.45	3.57 ± 0.55	3.87 ± 0.85
06	Trikatu churna	250	2.82 ± 0.25	4.00 ± 0.81	5.07 ± 0.09	4.50 ± 1.29
07	Analgin	40	4.25 ± 0.95	7.71 ± 0.91	9.42 ± 0.50	8.70 ± 0.14

Discussion

The increasing prevalence of multi drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections adds urgency to search for new infection fighting strategies. Several phytoconstituents like flavonoids and polyphenols (16), tannins (17), terpenoids (18), sesquiterpenes (19) have proven effective antimicrobial substances against a wide range of microorganisms.

The preliminary phytochemical observations of Trikatu churna and its individual ingredients have shown the occurrence of alkaloids, phenols, and essential oils, perhaps the combined effect of these groups of chemical compounds synergistically exhibited the inhibitory activity on the growth of bacteria and fungi. Since the analgesic activity is more connected to central nervous system, the active principles of the churna like piperine, gingerol, inoleresin and other active constituents of *Piper nigrum, P. longum,* and *Z. officinale* might directly act upon the central nervous system. Further detailed evaluation on the mechanism of action is needed. It is known that the commercially available analgesics causes irritability in the digestive system and also causes inflammation in allergic patients. Under such circumstances the Ayurvedic formulations such as churna which has carminative (20-21), and can also act as analgesic. This study would provide the preliminary scientific basis for the detailed phytochemical and pharmacological activity of such Ayurvedic preparations.

These above results also evidenced from the earlier reports on antimicrobial and analgesic activities of all the ingredients of Trikatu churna. The extract of *Z. officinale* rhizome produced significantly inhibition of the carrageenan-induced rat paw oedema and a reduction in the number of writhing induced by acetic acid in mice (22-25). Antimicrobial and analgesic activities were explored from *Piper* species (26-27). The *Piper nigrum* extract administered alone, showed significant decrease in writhes 78.43% with respect to control when *Piper nigrum* extract was co administered with diclofenac sodium. When Piper *nigrum* extract combined with pentazocine showed significant increase in tail flick latency in comparison with pentazocine alone and control group (28).

Conclusion

The extract of Trikatu churna was proved to be very effective against different bacterial and fungal isolates tested when compared to its individual ingredients. In fact these plants produce a wide range of bioactive molecules. Here, the combination of all these plant materials in equal proportions making the Trikatu churna preparation as one of the rich source of phytoconstituents and interaction of all these chemicals might be resulted in synergistically enhanced the therapeutic efficacy of antimicrobial and analgesic activities, thus lend pharmacological support to folkloric, ethnomedical and traditional uses of Trikatu churna for destruction of pathogenic microbes and increase the pain tolerance capacity in mice.

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