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Evaluation of antibacterial potential of Trikatu churna and its ingredients: An *in vitro* study

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Abstract

Herbal medicines are being used increasingly as dietary supplements to fight or prevent common disease. 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. The aqueous, ethanol, methanol and acetone extracts of these plant's fruits and Trikatu churna were prepared and antibacterial activities were tested by disc diffusion method against enteric bacterial pathogens such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus epidermidis, Salmonella typhi, Salmonella typhimurium* and *Enterobacter aerogenes.* The extracts of *Piper longum, Piper nigrum* and *Zingiber officinale* were found antibacterial to all bacterial pathogen tested. Trikatu churna exhibited potent antibacterial activity; this might be due to the multifunctional effect of all the three plant ingredients of Trikatu churna. Antibacterial activity of Trikatu churna and its ingredients was carried out in attempt to support the use of Trikatu churna for the treatment of enteric bacterial infections.

Keywords: Antibacterial activity, Trikatu churna, bacterial pathogens.

Introduction

India has an ancient heritage of traditional herbal medicine. With the emerging interest in the world to adopt to study the traditional system and to exploit their potentials based on different healthcare systems. World Health Organization [1] estimates that about 80% of the populations living in the developing countries are rely almost exclusively on traditional medicine for their primary health care needs [2]. The Trikatu churna is one of the classical Ayurvedic dosage form used in Ayurvedic system of medicine. It is official in ayurvedic formulary of India is combination of three reputed herbs, comprised of the fruits *Piper longum* L (Pippali), *Piper nigrum* L (Marica) and rhizomes of *Zingiber officianalis* Roscoe (Saunth). Trikatu churna is an Ayurvedic proprietary medicine containing Pipalli as an important constituent, which is used for bronchitis and asthma. All these plant materials are used world wide as spices. They are also used as important ingredients in folklore medicine in many Asian countries [3, 4]. However, the

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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 [5-8]. Of all the ingredients Pippali and Marica contain an alkaloid piperine as chief constituent [9]. *Piper longum* contain piperine, piper longamine, volatile oil, resin, gums and fatty oil. The fruits of *Piper longum* are useful in spleen disorders, bronchitis tuberculosis and jaundices [10]. Since the Piperine forms the major constitute of two of three ingredients Trikatu churna. Today there is wide spread interest in drugs derived from plants for their potential antimicrobial activity. Efforts are directed to identify plant product used in the treatment of various disease, which have broad spectrum antimicrobial properties [11]. Therefore the review revealed that the Trikatu churna were used in various metabolic disorders, but far their antibacterial properties were not demonstrated. The present study was under taken to evaluate the antibacterial activity of Trikatu churna and compared its effects to its individual ingredients against enteric bacterial pathogens.

Materials and Methods

Plant materials: The plant material *Piper nigrum, Piper longum* and rhizomes of *Zingiber officinale* were purchase from local market of Kopargaon and their identity was confirmed at Department of Botany, SSGM College of Science, Kopargaon, Dist Ahmednagar, India

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 [6].

Preparation of extracts: The aqueous extract was prepared by adding 20g of Trikatu churna and its ingredients (the powder of the dried fruits of *Piper nigrum, Piper longum* and rhizomes of *Zingiber officinale*) in 200mL distilled water, heated at 60^0C for 2h, filtered through cloth and the filtrate was evaporated on sand bath. The dry mass was then stored at 4^0C. The organic solvent extract was prepared by adding 20g of herbal preparation (powder) in 200 mL of organic solvent (acetone, ethanol and methanol) in screw-capped bottles; shake at 190-220 rpm on a rotary shaker. After 24h of shaking, the extract was filtered, evaporated in vacuum and dried by rotary evaporator at 60^0C [12]. Dried extracts were stored in labeled sterile screw capped bottles at 4^0C and later used for the in *vitro* study.

Phytochemical Screening of crude extracts: The presence of saponins, tannins, carbohydrates, alkaloids, flavonoids glycosides, steroids, proteins and alkaloids, were detected by simple qualitative methods [13, 14].

Preparation of disc for antibacterial activities: Aqueous, ethanol, methanol and acetone extracts of Trikatu churna (Powder) and its ingredient were prepared in their respective solvents and the sterile blotting paper disc (10 mm) were soaked in the diluted extract in such concentration that the amount of solution absorbed by each disc was 5mg of each extracts. The prepared disc were dried in controlled temperature to remove excess of solvent and used in study.

Bacterial cultures: The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The bacterial cultures were rejuvenated in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) at 37^0C for 18h and then stocked at 4^0C in Mueller-Hinton Agar. The inoculum size of the bacterial culture was standardized according to the National committee for Clinical Laboratory Standards guideline [15]. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 37^0C for 3h until the culture attained a turbidity of 0.5 McFarland units. The final inoculums size was standardized to 10^5 CFU/mL with the help of SPC and Nephlo-turbidometer.

Table 1: Bacterial cultures used in study (IMTECH, Chandigarh, India).

Bacterial Pathogens	MTCC Number
Proteus vulgaris	426
Staphylococcus epidermidis	435
Staphylococcus aureus	96
Escherichia coli	739
Pseudomonas aeruginosa	424
Klebsiella pneumoniae	109
Salmonella typhi	733
Enterobacter aerogenes	111
Salmonella typhimurium	98

Antibacterial activity using disc diffusion method: The modified paper disc diffusion was employed to determine the antibacterial activity of aqueous, ethanol, methanol and acetone extracts of Trikatu churna. Turbidity of inoculums was matched with McFarland turbidity standard. Inoculums were spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth. Then the prepared antibacterial disc were placed over the lawn and pressed slightly along with positive and negative controls. Ampicillin 10mcg/disc (Hi-Media, Mumbai) were used as positive control

Table 2: Antibacterial activity of Trikatu churna and its ingredients against enteric bacterial pathogens at 5mg/disc (Zone of inhibition of growth in mm, average of 3 readings)

Medicinal plant	Solvent extract	*S. epidermidis*	*S. aureus*	*P. vulgaris*	*P. aeruginosa*	*S. typhi*	*E. coli*	*K. pneumoniae*	*S .typhimurium*	*E. aerogenes*
Piper nigrum	Aqueous	15	14	12	-	-	12	-	-	-
	Ethanol	17	16	14	12	12	14	12	12	-
	Methanol	18	17	16	14	15	15	14	14	13
	Acetone	23	22	18	17	17	17	16	16	17
Piper longam	Aqueous	15	16	13	12	-	12	13	-	13
	Ethanol	17	17	15	15	13	14	13	12	15
	Methanol	20	18	16	16	15	17	15	15	18
	Acetone	23	22	17	18	17	20	18	17	20
Zingiber officinale	Aqueous	14	15	13	12	13	12	12	12	0
	Ethanol	15	17	15	14	15	14	15	13	18
	Methanol	18	20	17	16	17	16	17	15	15
	Acetone	22	24	20	17	20	18	19	18	20
Trikatu churna	Aqueous	15	14	13	12	13	12	13	12	14
	Ethanol	17	16	16	15	15	15	15	15	16
	Methanol	21	20	17	17	18	18	18	17	17
	Acetone	25	24	20	19	20	20	20	19	21
Negative control	Aqueous	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	-	-	-
	Acetone	-	-	-	-	-	-	-	-	-
Positive control	Ampicilin (10mcg)	25	24	16	16	18	11	18	19	14

Table 3: Phytochemical screening of crude extract of 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	+	+	+	+

while disc soaked in sterile distilled water or various organic solvents and dried were placed on lawns as negative control. The plates were incubated for 18h at 37^0C. The antibacterial activity was evaluated and diameter of inhibition zones was measured. Experiment was carried out in triplicate and the averages diameter of zone of inhibition was recorded. The antibacterial activity was classified as strong (>20mm), moderate (15-19mm) and mild (13-14mm) and less than 12mm was taken as inactive.

Results and Discussion

The present study demonstrated that, the aqueous and alcoholic extract of Trikatu churna and its individual ingredients possess potent antibacterial activity (Table 2). The preliminary phytochemical observations of crude 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 3). 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 antibacterial activity.

According to the antibacterial profile of Trikatu churna and its ingredients (Table 2), it was observed that the acetone extract of *Piper nigrum* was strong antibacterial against *S. epidermidis* and *S. aureus,* while moderate against *P. vulgaris, P. aeruginosa, S. typhi, E. coli, K. pneumoniae, S. typhimurium* and *E. aerogenes,* whereas aqueous extract was mils against *P. vulgaris, S. aureus* ethanol extract was mild against *P. aeruginosa, S. typhi* methanol extract mild antibacterial against *E. aerogenes.* It was also reported as antibacterial against *E. aerogenes, K. pneumoniae* and *P. mirabilis* [12]. Methanol extract of *Piper longum* was strong antibacterial against *S. epidermidis* and moderate antibacterial against *P. vulgaris, P. aeruginosa, S. typhi, E. coli, K. pneumoniae, S. typhimurium, E. aerogenes,* while acetone extract was strong antibacterial against *S. epidermidis , S. aureus, E. coli* and *E. aerogenes* and moderate antibacterial against *P. vulgaris, P. aeruginosa, S. typhi, K. pneumoniae* and *S. typhimurium. Piper longum* was reported as a strong antibacterial against *B. cereus* and *E. coli* [16], which is traditionally used for chronic bronchitis, asthma [17].

Acetone extract of *Zingiber officinale* was strong antibacterial against *S. epidermidis, S. aureus, P. vulgaris, S. typhi, E. aerogenes* and moderate

antibacterial against *P. aeruginosa, E. coli, K. pneumoniae, S. typhimurium,* whereas aqueous and ethanol extract was mild antibacterial against *S. epidermidis , P. vulgaris, P. aeruginosa, S. typhi, E. coli, K. pneumoniae, S. typhimurium, E. aerogenes. Zingiber officinale* has been showed to have antimicrobial activity [18]. Antibacterial activity of Trikatu churn showed that ethanol extract was moderate antibacterial against all the bacterial pathogens while methanol extract was strong antibacterial against *S. epidermidis* and *S. aureus* moderate antibacterial against *P. vulgaris, P. aeruginosa, S. typhi, E. coli, K. pneumoniae, S. typhimurium, E. aerogenes* whereas acetone extract was strong antibacterial against *S. epidermidis, S. aureus, P. vulgaris, S. typhi, E. coli, K. pneumoniae, E. aerogenes* and moderate antibacterial against *P. aeruginosa* and *S. typhimurium.*

There are various reports in the literature regarding the antibacterial properties of individual ingredients of Trikatu churna; *Piper longum* is well known for its immuno modulator action and rejuvenating effect on digestive and respiratory system and it is antibacterial against *B. megaterium, S. aureus, S. ß-haemolyticus, B. subtilis, S. lutea, E. coli, P. aeruginosa, S. sonnai, S. dysenteriae, S. typhi,* and antifungal against *A. nigar, A. fumigatus,* and *C. albicans* [19]. It was observed that the Trikatu churna possess wide range of antibacterial properties against *S. epidermidis, S. aureus, P. vulgaris, B. subtilis, P. aeruginosa,* and *K. pneumoniae* and could be useful against above test bacterial infections. 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 antibacterial 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 antibacterial activity. It is also confirmed that, these spicy products triggers natural immune system to fight against enteric bacterial infection. This study would provide the preliminary scientific evidence for ethno-botanical and traditional use of this churna for prevention of enteric bacterial infections.

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References

1. WHO, General guidelines for methodologies on research and evaluation of traditional medicine, World Health Organization, Geneva, 2000.
2. Parle M, Bansal N. Herbal Medicine: Are they safe? Nat Prod Red, 2005; 5(1):6-16.
3. Anonymous. Indian Pharmacopoeia, Government of India, III-Edition, New Delhi, Appendix IV, 1985; 90.
4. Chopra RN, Nayar SL, Chopra IC, In Glossary of Indian Medicinal Plants, Publications and Information Directorate, CSIR, New Delhi, 1992; 132-210.
5. George Watt. In a dictionary of the economic products of India, Periodical experts, 42-D, Vivek Vihar, Delhi, 1972; 217-247.
6. Lodha R, Kabra SK. Bronchial asthama In Scientific Basis For Ayurvedic Therapies, Edited by Lakshmi Chandra Mishra., Published by CRC Press, 2003.
7. Namjoshi AM. Ayurvedic formulary of India, Controller of Publications, Delhi, 1976; 85-95.
8. Sivarajan VV, Indira Balachandran. Ayurvedic drugs and their plant sources. Published by Mohan Primalini for Oxford

and IBH Publishing Company, PVT. LTD, New Delhi, 1996.

9. Kokate CK, Chaudhari GN, Nimbkar AY. Search for anthelmintics of plant origin: activities of volatile principles of *Acorus calamus* and *Piper longum* against Ascaris lumbricoides, Asian Symposium on Medicinal Plants and Spices, Conference, Bangkok (Thailand), 1980; 15-19.
10. Kiritikar KR, Basu BD. Indian medicinal plants. 2nd edn. International book distribution Vol. III. Dehradun, 1987; 1664–1666.
11. Parekh J, Nair R, Chanda S. Preliminary screening of some folkloric plants from Western India for potential antimicrobial activity. Indian J. Pharmacol. 2005; 37: 408-409.
12. Parekh J, Chanda S. In vitro screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae. Afr J Microbiol Research. 2007; 1(6):92-99.
13. Harborne JB. Phytochemical Methods. A guide to modern techniques of plant analysis, 3rd Edn. Chapman and Hall, London, 1998; 235.
14. Khandelwal KR. Preliminary photochemical screening, in: Practical Pharmacognosy Techniques and Experiments. 149-156, 8th edn. Nirali Publication, Pune, 2001.
15. NCCLS (National Committee for Clinical Laboratory Standards), 2002. Performance Standards for antimicrobial susceptibility testing. 8th Informational Supplement. M100 S12. National Committee for Clinical Laboratory Standards, Villanova, Pa, 2002.
16. Elizabeth and Williamsons, Major herbs of Ayurveda, Churchill living stone, Elsevier Science Ltd, London, 2002.
17. Kapoor LD. Handbook of ayurvedic medicinal plants. 1st Edn, CRC Press Boca Raton (FL), 1990; 17-342.
18. Srinivasan D, Nathan S, Suresh T, Perumalsamy O, Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J Ethnopharmacol, 2001; 74:217-220.
19. Ali MA, Alam MN, Yeasmin SM, Khan AM, Sayeed MA, Antimicrobial screening of different extracts of *Piper longum* Linn. Res J Agri Biol Sci, 2007; 3(6):852-857.