Research Article

Available online through
www.ijrap.net

IJRAP
ISSN 2229-3566

PHARMACOGNOSTICAL AND PHYTOCHEMICAL SCREENING OF TRIKATU HERBS THE HEALING TOUCH OF AYURVEDA

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Received on: 11/06/2011 Revised on: 23/07/2011 Accepted on: 10/08/2011

ABSTRACT
Ayurveda, arguably the oldest system of rnedicine in the world, frequently uses fixed combinations of herbs. An important ingredient of many recipes, some of which date back to 6000 BC, is 'Tlikatu' (Sanskrit, meaning 'three acrid'). Which is a mixture of black pepper *Piper nigrum*; long pepper, *Piper longum*; and ginger, *Zingiber ofticinale*; the reason for the inclusion of these has recently been examined and a theory for their use proposed which involves enhancement of bioavailability. The bioavailability enhancement probably results from the fact that piperine is a poter inhibitor of drug metabolism.
KEYWORDS: *Zingiber ofticinale, Piper longum, Piper nigrum,* Tlikatu, pharmacognostical study, Piperine.

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INTRODUCTION

Medicinal plants have shown tremendous potential for the development of the new drug molecules for various serious diseases. Many plant derived products have found to play an important role in various disease conditions. Although, modern medicines are available, herbal medicines have often retained popularity for historical and cultural reasons. Since the usage of these herbal medicines has increased, the issues regarding their safety, quality, and efficacy in industrialized and developing countries are cropped up[1].

The chemistry of *Zingibet ofticinale* is well documented with respect to the oleo-resin and volatile oil. Oleo-resin components are considered to be the main active principles in ginger and documented pharmacological actions generally support the traditional uses. In addition, a number of other pharmacological activities have been documented, including hypoglycaemic, antihypercholesterolaemic, anti-ulcer and inhibition of prostaglandin synthesis, all of which require further investigation. The use of ginger as a prophylactic remedy against motion sickness is contentious. It seems likely that ginger may act by a local action on the gastrointestinal tract, rather than by a centrally mediated mechanism[2].

Piper longum Linn is pitta suppressant and vata and kapha aggravator. It has a strong erge to suppress any kind of infection occurring in the body due to its pungent taste. It helps in en expelling out the mucus that gets accumulated in the respiratory tract and also the sinuses present in face due to its sharp properties. Fruits—used for diseases of the respiratory tract (cough, bronchitis, asthma) It also strengthens the nervous system. It is a good digestive agent and helps in improving the gastrointestinal condition and also normalizes the peristaltic movements. It has a great effect on the respiratory tract[3] . Piperine enhanced bioavailability of hexobarbital, phenytoin, propranolol and theophylline. (Sharon M. Herr.) (Piperine is also a component of Piper nigrum.) N-isobutyl-deca-trans-2-trans-4-dienamide, isolated from the fruit, exhibited antitubercular property. Milk extract of the fruit effectively reduced passive cutaneous anaphylaxis in rats. It protected guinea-pigs against antigen-induced bronchospasm.

Piper nigrum Linn used as Stimulant, carminative, diuretic, anticholerin, sialagogue, and bechic, antiasthmatic. Used in, fevers, dyspepsia, flatulence, indigestion, and as mucous membrane and gastro-intestinal stimulant. Pepper was once employed in the treatment of gonorrhoea and chronic bronchitis

externally— rubefacient and stimulant to the skin. Used as a gargle for sore throat. Used with ginger and Piper longum for viral hepatitis. The fruit yielded piperine, piperatine and piperidine; amides, piperyline, piperoleins A and B, and N-isobutyl- cicosa-trans-2-trans-2-dienamide[4].

Definition

Trikatu Curna is a fine powder preparation made with the following composition: (Table 1)

Trikatu is beneficial in following conditions:

1. Trikatu is a very is a powerful stimulant for both the digestive as well as respiratory systems. Because of its strong heating affects it brings about the removal of cold, congestion, and revives weak organic functions.
2. Trikatu acts as rejuvenate for the lungs and Kapha.
3. Trikatu is also known to be having aphrodisiac properties strengthening the reproductive functions, warming and energizing the reproductive organs. It is because of this known affects of Trikatu that it helps in increasing the sperm count and helps in the maintenance of a healthy sexual life.
4. Trikatu is an anti-mucus and digestive powder used to improve gastric and respiratory function.
5. Trikatu is also useful in cases of obesity, weak digestion, high cholesterol, high triglycerides, hypothyroid, slow metabolism, congestion, cough, and edema.
6. Trikatu brings about the reduction of Kapha (water and earth) and increases Pitta (fire) through the rejuvenation of low Agni (fire) and the burning away of Ama (toxins). This is the reason it is strongly recommended for the treatment of cold, congestion, reviving weak organic functions.
7. Trikatu is a safe digestive stimulant and expectorant.
8. It is antiallergenic, carminative, anti - flatulent, and acts as a natural antihistamine.
9. Trikatu is a very effective in treatment of dyspepsia; it provides a balanced heat to warm digestion and circulation and to prevent the formation of gastric mucosa.
10. Trikatu is used internally in the treatment of gastric and abdominal disorders, asthma, bronchitis, coughs, dysentery, pyrexia, and insomnia.
11. When Trikatu is administrated it promotes the secretion of digestive juices, increasing appetite. Trikatu stimulates the formation of hydrochloric acid. It helps to inhibit gaseous distension.
12. It is prescribed as an essential part of many multi-herb preparations, as it has been shown to increase the bioavailability of nutrients, foods, and medicines.
13. It promotes rapid absorption of nutrients by the gastrointestinal tract.
14. Its thermogenic effect, metabolic enhancement, and nutrient bioavailability enhancing properties render Trikatu particularly beneficial in the support of respiratory health.
15. Trikatu is also a best anti obesity drug. It helps the body to get rid of excessive fat and cholesterol.
16. Trikatu helps to reduce down the levels of cholesterol and restores the lipids in the body.
17. Trikatu is also useful in pain and inflammations.

MATERIALS AND METHODS

The fresh healthy plant materials Zingiber *officinale, Piper longum, Piper nigrum* were collected from various domestic places West Bengal. Then the fresh herbs were taken and washed properly with raw water and finally with Purified water, then Microtome sections were cut and stained with safranin and fast green and photographed with Nikon F70X camera. After that the herbs were taken to mixer grinder for proper grinding and pasting of which was finally transferred to juicer for collection of juices. The juices were used for different tests and analysis.

RESULTS

Macroscopic and microscopic analysis

The macroscopic characters such as colour, odour, taste, nature, texture were studied for morphological investigation. The quantitative microscopy was studies as per the procedure given in "practical pharmacognosy" by C. K. Kokate.

Macroscopy

Sunth (*Zingiber officinale*)

Rhizome laterally compressed, bearing short, flattened, oblique branches; outer surface buff-coloured, longitudinally striate; inner surface pale yellow, starchy and fibrous. Fracture short with projecting fibers[6].

Pipul (*Piper longum*)

Fruit - greenish-black to black, cylindrical, 2.5 to 5 cm long and 0.4 to 1 cm thick, arranged around an axis; surface- rough and composite; broken surface shows a central axis and 6 to 12 fruitlets arranged around an axis; taste- pungent producing numbness on the tongue; odour- aromatic[7].

Marich *(Piper nigrum)*

The fruits are globular or oblong, 4-6 mm in diameter. The outer cover is blackish brown, with raised reticulated wrinkles. One seeded, seeds are white and hollow[8].

Microscopy

Sunth (*Zingiber officinale*)

Fibro-vascular bundles and oleoresin cells with yellow pigment scattered in ground tissue. Starch grains

abundant in parenchyma cells, mostly simple, sack shaped, spherical, and hilum eccentric[6].

Pipul (*Piper longum*)

Catkin shows 6 to 12 fruits, arranged in circle on a central axis, outer epidermal layer of irregular cells filled with deep brown content and covered externally with a thick cuticle; mesocarp consists of larger cells, usually collapsed, irregular in shape and thin-walled; a number of stone cells in singles or in groups present; endocarp and seed coat fused to form a deep zone, outer layer of this zone composed of thin-walled cells and colourless, inner layer composed of tangentially elongated cells, having reddish-brown content; endocarp filled with starch grains[7].

Marich (*Piper nigrum*)

The fruit has a well differentiated pericarp, testa and perisperm. Isolated, tangentially elongated oil cells are in the outer region of the mesocarp. Endocarp has beaker shaped stone cells and numerous polyhedral masses of starch Grains. Test has a single layer of yellow colored cells[8].

PHYSICAL EVALUTION

pH Determination

Procedure

60ml. of fresh juice extracts are filtered properly then pH of the filtrates were checked with a pH meter (Elico) having standardized glass electrode[9]. (Table No. 2)

Volatile Oil Determination

Procedure

5.0ml of plant extract and 200ml of water was taken in a Clevenger apparatus and distilled for 2 hours. 0.1 ml. of volatile oil was separated out during this period, indicating the presence of volatile oil. (Table No. 3)

QUALITATIVE CHEMICAL EVALUATION[10 11 12]

All the fresh juices were subjected to qualitative chemical tests. (Table No. 4)

Test for Alkaloids

Small amount of solvent free extract was stirred with a few ml. of dilute hydrochloric acid and filtered.The filtrates were tested with various alkaloidal reagents such as Mayer's, Dragen Dorff's, Wagner's and Hager's reagent, phosphomolybdic acid and tannic acid. Formation of characteristic colour precipitated were preserved.

Test for Glycosides

Small amount of the extract was dissolved separately in 5ml of distilled water and filtered.

Another portion of the extract was hydrolyzed with hydrochloric acid for one hour on a water bath and hydrolysate was subjected to Legal's, Baljet's, Borntrager's, Keller-Kiliani's tests and for the presence of cyanogenic glycosides.

Legal's Test

To the hydrolysate, 1ml of pyridine and a few drops of sodium nitroprusside solution was added and then made alkaline with sodium hydroxide solution. Formation of pink colour confirms the presence of glycosides.

Baljet's Test

To a section of leaf sodiumpicrate solution was added. Observation of yellowish orange colour confirms the presence of glycoside.

Borntrager's Test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal volume of dilute ammonia solution was added. Formation of pink colour in ammoniacal layer confirms the presence of glycoside.

Test for Cardiac Glycosides [Keller – Kiliani Test]

To the extract was, 10ml of 75% alcohol were added, boiled on water both, filtered. The filtrates were diluted with distilled water; 1ml of strong lead acetate solution was added and filtered. The filtrates were extracted with an equal volume of chloroform. The chloroform layer was pipette out and evaporated to dryness. The residue obtained was dissolved in 3ml of 3.5% of ferric chloride in glacial acetic acid, it was left for 1 minute and then trams referred to a test tube. To the side of test tube 1.5ml of sulphuric acid was added carefully. Formation of brown colour at the interface and pale green colour in upper layer confirm the presence of digitoxose.

Test for Phytosterols

The extract was refluxed with 0.5N alcoholic potassium hydroxide until the saponification was complete. The saponification mixture was diluted with distilled water and extracted with petroleum ether.The ethereal extract was evaporated and unsaponifiable matter was subjected to Liebermann's, Liebermann's- Burchard's and Salkowski's test.

Liebermann's Test

The residue was dissolved in concentrated sulphuric acid and a few drops of aqueous sodium nitrate were added. Formation of red colour on dilution and turned to blue when made alkaline with aqueous sodium hydroxide confirm the presence of phytosterol.

Liebermann – Burchard's Test

The residue was dissolved in chloroform. To this Liebermann – Burchard's reagent was added. Formation of green colour confirms the presence of phytosterol.

Salkowski's Test

A few drops of concentrated sulphuric acid were added to chloroform solution. Formation of brownish red colour in the lower layer confirms the presence of phyto chemical test.

Test for Flavonoids

Different extract was separately dissolved in ethanol and then subjected to the following tests.

Ferric Chloride Test

To a small quantity of the ethonolic solution, few drops of neutral ferric chloride were added. Formation of blackish red colour confirms the presence of flavonoids.

Shinoda's Test

To the alcoholic solution a small piece of magnesium ribbon was added along with concentrated hydrochloric acid. Formation of magenta colour confirms the presence of flavonoids.

Fluorescence Test

Alcoholic solutions were seen under UV light. Observation of green fluorescence was shown.

Reaction with Acid and Alkali

When alcoholic solutions were treated with alkali gave yellowish green colour which on addition of acid become colour less.

Test for Tannins and Phenolic Compounds

The extract was dissolved in distilled water and filtered. The filtrates were treated with various reagents.

Few ml. of the filtrates were treated with 5% ferric chloride solution. Observation of bluish black colour confirms the presence of phenolic compound.

Few ml of the extract was treated with lead acetate solution. Observation of white precipitate confirms the presence of tannins.

Few ml of the filtrates were treated with strong potassium dichromate solution. Formation of precipitate confirms the presence of tannins.

Few ml of the extract was treated with potassium ferricyanide followed by ammonia. Formation of deep red colour confirms the presence of phenolic compounds.

Test for Saponins

Foam Test

The extract was diluted with 20ml. of distilled water and agitated in a graduated cylinder for 15mins. Formation of 1cm layed foam confirms the presence of saponins.

Analytical works on Piperine

UV spectral analysis

5µg/ml of the drug in ethanol was used for complete scan between 190-900nm and the maximum absorption was obtained at 344nm as shown in the fig.1 whereas the λ max for standard Piperine is 342nm[13].

DISCUSSION

Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. Before any drug can be included in the pharmacopoeia, these standards must be established. The majority of the information on the identity, purity and quality of the plant material can be obtained from its macroscopy, microscopy and physio– chemical parameters. From the above studies fruit of *Zingibet ofticinale, Piper longum, Piper nigrum,* can be easily differentiated on the basis of organoleptic and microscopical characters. Physic-chemical values viz.Alkaloids, Glycosides, phytosterol, Flavonoids, Saponins, and tannin were determined by using standard method and techniques. The above studies provide information in respect of their identification, chemical constituents & physicochemical characters which may be useful for pharmacognostical study and standardization of herbal drugs of folk medicinal practice of present era and enrichment of ayurvedic pharmacopoeia. It will also determine therapeutic diagnostic tools for the scientists who are keen and sincere to evaluate the herbal medicine of indigenous resources.

ACKNOWLEDGEMENT

The authors express their gratitude to Gayatri College of Pharmacy, Department of Pharmacognosy, Sambalpur, Orissa-768200, India for the support throughout the project is gratefully acknowledged.

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TABLE 1: COMPOSITION OF TRIKATU CHURNA PREPARATION

S.No	Ingredients	Botanical Name	Part used	Quantity used (gm)
1	Sunthi	*Zingiber officinale* Rosc	Rhizome	500
2	Pippali	*Piper longum* Linn	Fruit	500
3	Marica	*Piper nigrum* Linn	Fruit	500

TABLE 2: pH OF FRESH JUICE EXTRACTS

	Sunth (*Zingiber officinale*)	Pipul (*Piper longum*)	Marich (*Piper nigrum*)
pH of fresh juice	4.93	6.76	6.67

TABLE 3: VOLATILE OIL OF THE FRESH JUICE EXTRACTS

	Sunth (*Zingiber officinale*) in %	Pipul (*Piper longum*) in %	Marich (*Piper nigrum*) in %
Volatile oil contains	2%	1-2.5%	1-2%

TABLE 4: TESTS WITH JUICE EXTRACTS

	Sunth (*Zingiber officinale*)	Pipul (*Piper longum*)	Marich (*Piper nigrum*)
TEST FOR ALKALOIDS			
Dragendroff's test	+	+	+
Mayer's test	+	+	+
Wagner's test	+	+	+
Hager's test	+	+	+
TEST FOR GLYCOSIDES			
Legal's test	–	–	–
Baljet's test.	–	–	–
Borntragers test.	–	–	–
Keller-killiani's test.	–	–	–
TEST FOR PHYTOSTEROLS			
Liebermann's test .	+	+	+
Liebermann-Burchard'stest	+	+	+
Salkowski's test	+	+	+
TEST FOR FLAVONOIDS			
Fecl3 test	+	+	+
Shinoda's test	–	–	–
Fluorescence test	+	+	+
Zinc hydrochloride test	–	–	–
TEST FOR TANNINS &PHENOLIC COMPOUNDS			
5% FeCl3 solution	–	–	–
Reaction with lead acetate	+	–	–
Reaction with potassium dichromate	–	–	–

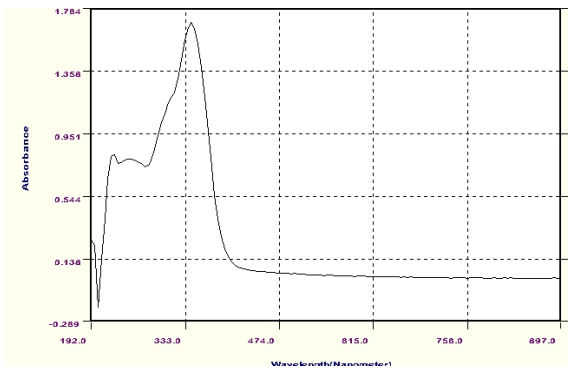

Fig. 1: Absorption maximum of Piperine

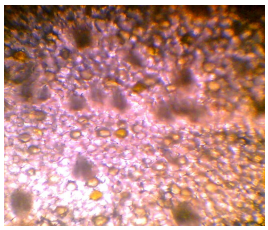

Fig.1: T.S OF *ZINGIBER OFFICINALE*

Fig.2: Ginger – dried drug (rhizome).

Fig.3: T.S OF *PIPER NIGRUM*

Fig.4: Marich-dried drug (fruits)

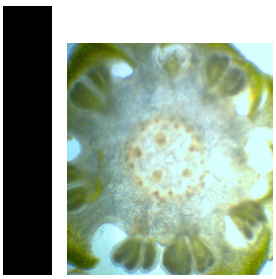

Fig.5: T.S OF *PIPER LONGUM*

Fig.6: Pipul- fresh drug(fruits)