Comparative Evaluation of Antimicrobial Property, Pharmacological and Toxicological Studies of Amoxicillin and Pawitra Tulsi Gold

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ABSTRACT

Pawitra Tulsi Gold is a mixture of 7 types of Basil named Ocimum basilicum, Ocimum sanctum, Ocimum canum Simpson, Ocimum gratissimum, Ocimum kilimandscharicum, Lallemantia royleana and Ocimum lemon. Objective of this study is to find the antimicrobial property of “Pawitra Tulsi Gold” and safety in animal model.

Keywords: Amoxicillin, Tulsi, Streptococcus, E.Coli.

INTRODUCTION

Streptococcus and E.coli infection is increasing in Indian subjects and lungs diseases are important risk factors. Both Gr+ve and Gr-Ve infections are increasing in India contrast to developed countries of Europe and North America. The use of antibiotics is increasing in a rapid rate. Solution for both Intention of this study is to find out some suitable herb Gr+ve and Gr-Ve infections.

MATERIALS

Seven varieties of Tulsi used in Tulsi gold

Ocimum basilicum

Plant description

Basil grows between 30–130 cm tall, with opposite, light green, silky leaves 3–11 cm long and 1–6 cm broad. The flowers are small, white in color and arranged in a terminal spike. Unusual among Lamiaceae, the four stamens and the pistil are not pushed under the upper lip of the corolla, but lie over the inferior lip.

Habitat

Basil is originally native to India and other tropical regions of Asia, having been cultivated there for more than 5,000 years.

Folk Use

Basil is used for their medicinal properties in Ayurveda, the traditional medicinal system of India and Siddha medicine, a traditional Tamil system of medicine. They are also used as drinks in Southeast Asia.

Chemical components

Other chemicals that help to produce the distinctive scents of many basils, depending on their proportion in each specific breed, include: Citronellol, Linalool, Myrcene, Pinene, Ocimene, Terpineol, Linalyl acetate, Fenyl acetate, Trans-ocimene, 1,8-cineole, Camphor octanane, Methyl eugenol, Eugenol & Beta-caryophyllene.
Potential health effects

Recently, there has been much research into the health benefits conferred by the essential oils found in basil. Scientific studies in vitro have established that compounds in basil oil have potential antioxidant, antiviral, and antimicrobial properties, and potential for use in treating cancer. In addition, basil has been shown to decrease the occurrence of platelet aggregation and experimental thrombus in mice. It is traditionally used for supplementary treatment of stress, asthma and diabetes in India. In Siddha medicine, it is used for treating pimples on the face, but noted that intake of the seeds in large quantities is harmful for the brain.

Basil, like other aromatic plants such as fennel and tarragon, contains estragole, a known carcinogen and teratogen in rats and mice. While human effects are currently unstudied, extrapolation using body weight from the rodent experiments indicates that 100–1000 times the normal anticipated exposure still probably produces a minimal cancer risk.

Ocimum sanctum

Sanskrit synonyms
Tulasi, Surasa, Svetatulasi.

Ayurvedic properties
Rāṣa : Kashaya, Tikta
Guna : Lakhu, Rooksha
Virya : Ushna

Plant description
An erect much branched undershrub, grows up to 1 m in height. Leaves are pale dark brown in color, simple, opposite, elliptic, oblong, obtuse or acute, serrate, entire, pubescent on both sides. Petiole slender and hairy. Flowers purplish in elongate recemes. Fruits nutlets, smooth, not mucilaginous when wet.

Medicinal properties
Plant pacifies vitiated tridoshas, cough, asthma, bronchitis, fever, toxins, vomiting, lumbago, gastric distension, genito-urinary diseases, ringworm and skin diseases.

Useful part: Whole plant.

Therapeutic applications
Cardiovascular activity, Chemoprotective activity, Hypoglycemic activity, Hypotensive activity, Wound-healing activity

Ocimum canumsims

Family
Lamiaceae
Synonym
O. americanum Linn.

Family
Labiatae; Lamiaceae.

Habitat
Plains and lower hills of India.

English
Hoary Basil.

Ayurvedic
Kaali Tulasi, Vana-Tulasi.

Siddha/Tamil
Ganjamkorai, Nai-Tulasi.

Action
Plant—stimulant, carminative, diaphoretic. Leaf—bechic, febrifuge; used in cold, bronchitis, catarrh, externally in skin diseases. Essential oil—antifungal. Seeds—hypoglycaemic; also used in the treatment of leucorrhoea and other diseases of urinogenital system.

Chemical Constituents
The essential oil at the flowering stage contains citral as a major component along with methylheptenone, methylnonylketone and camphor. Leaves yielded beta-sitosterol, betulinic acid and ursolic acid and flavonoids, pectolinarigenin-7-methylether and nevadesin.

Local Names
English (wild basil, tree basil, East Indian basil, clove basil).

Botanic description
Ocimum gratissimum is an aromatic, perennial herb, 1-3 m tall; stem erect, round-quadrangular, much branched, glabrous or pubescent, woody at the base, often with epidermis peeling in strips. Leaves opposite; petiole 2-4.5 cm long, slender, pubescent; blade elliptical to ovate, 1.5-16 cm x 1-8.5 cm, membranaceous, sometimes glandular punctate, base cuneate, entire, margin elsewhere coarsely crenate-serrate, apex acute, puberulent or pubescent.

Medicinal uses
The whole plant and the essential oil have many applications in traditional medicine, especially in Africa and India. Preparations from the whole plant are used as stomachic and in treating sunstroke, headache and influenza. The seeds have laxative properties and are prescribed against gonorrhoea. The essential oil is applied against fever, inflammations of the throat, ears or eyes, stomach pain, diarrhoea and skin diseases. It is being tested as an antibiotic.

Chemical composition
The chemical composition of the oil is variable and at least 6 chemotypes have been reported, characterized by the main component of the essential oil: eugenol, thymol, citral, ethyl cinnamate, geraniol and linalool. The eugenol-type oil is a brownish-yellow to pale yellow liquid with a powerful, warm-spicy and aromatic odour, reminiscent of clove oil, but with a sweet-woody, almost floral top note. The thymol-type oil is a dark yellow to orange-yellow or brownish liquid with a medicinal-spicy, warm and somewhat herb-like odour. Its flavour is warm, slightly astringent and burning, and has a sweet medicinal aftertaste. Its colour is warm, slightly astringent and burning, and has a sweet medicinal aftertaste. The concrete obtained by solvent extraction is much richer in thymol than the distilled oil. A geraniol-rich type, found in the United States, contained mainly geraniol (84-88%) with small amounts of gamma-muurolene, neral, beta-caryophyllene and limonene. The citral type, reported from India is rich in citral (67%) and geraniol (26%).

Ocimum gratissimum
**Ocimum kilimandscharicum**

**Synonym**
African blue basil.

**Plant Description**
It is one of the most cold-tolerant breeds of basil, leading to it being called a perennial, though in fact all basils are perennial as long as the weather is warm year-round. It is a sterile hybrid of two other breeds of basil, unable to produce seeds of its own, and is propagated by cuttings. The leaves of African blue basil start out purple when young, only growing green as the given leaf grows to its full size, and even then retaining purple veins. This breed is also taller than many basil cultivars.

**Chemical Constituents**

Aqueous extract of leaves of *Ocimum contains* camphor, 1,8-cineole, limonene, trans caryophyllene, camphene, 4-terpeneol, myrtenol, α-terpineol, endo-borneol, linalool. Leaves also contain flavonoids, tannins, saponins, sterols, carbohydrates, proteins and triterpenoids. These chemical constituents are mainly responsible for various biological activities.

**Traditional Activities**
In traditional medicine, this plant is widely used for the treatment of various ailments including colds, coughs, abdominal pains, measles and diarrhoea. The leaves treat congested chest, cough and cold, by sniffing crushed leaves or inhaling vapour of boiling leaves. Infusion of leaves is a cure for measles Essential oils posses biologically active constituents that act as insect repellents, particularly against mosquitoes and storage pests. Some local farmers also mix stored foodstuffs with dry leaves of *Ocimum kilimandscharicum* for protection against insect pest damage in storage. It show antibacterial and antioxidant activity. It also used in viral infections, foul ulcers, anorexia and for healing wounds. *Ocimum kilimandscharicum* in boiled water in a pot or saucepan to generate an aroma. It is also used in the Mediterranean area in interesting forms for decorative purposes.

**Pharmacological Activities**
Antioxidant activity, Antimicrobial activity, Wound healing activity, Antibacterial activity, Antifungal activity

**Lallemantia royleana**

**Family**
Labiatae.

**Habitat**
Plain and hills of Kumaon and Punjab, extending westwards to Afghanistan. Imported into India from Persia.

**Action**
Seed-cooling, diuretic, sedative; given internally as a soothing agent.
**sOcimum lemon**

**Synonym**
Lemon basil, Thai lemon basil or Lao basil.

**Habitat**
The herb is grown primarily in northeastern Africa and southern Asia for its strong fragrant lemon scent, and is used in cooking.

**Plant Description**
Lemon basil stems can grow to 20–40 cm tall. It has white flowers in late summer to early fall. The leaves are similar to basil leaves, but tend to be narrower. Seeds form on the plant after flowering and dry on the plant.

**Photographs of Toxicological & Pharmacological Studies of Tulsi Gold**

![Fig. 1: Colony of mice](image1)

![Fig. 2: Oral administration(i.p.) of TULSI GOLD](image2)

![Fig. 3: Collection of blood from heart by cardiac puncture](image3)
TOXICOLOGICAL STUDIES OF TULSI GOLD

TOXICITY STUDY
In the evaluation of the toxic characteristics of a substance, determination of acute oral toxicity is an initial step. It provides information about a number of health hazards likely to arise from short-term exposure by the oral route. Data from an acute toxicity study may serve as a basis for classification, labeling and also in establishing a dosage regimen in sub-chronic and other studies. They also may show good therapeutic activity. Thus it helps in the determination of minimum dose, which can produce desired results in 50% population.

In any toxicity experiment animals are treated with drugs and observed for toxic manifestations. To increase the chances of toxic manifestations the dose is chosen much higher than the therapeutic dose and for a longer duration. Drug is administered to a group of animals to get statistically reliable results.

For acute toxicity study a group of animals are taken comprising of 5-10 animals in each group. Generally mice or rats are employed for the study. Sometimes dogs or monkeys are also used for this purpose. However, determination of therapeutic dose, emetic dose, and minimum symptomatic or toxic dose is greatly encouraged as these give ideas about the extent of toxic manifestations when given at a particular dose.

The animals used for the acute toxicity study are kept in identical laboratory condition at least two weeks for acclimatization to the working environment. They are fasted for 18 hours prior to drug administration. The dose should be such that at least three doses should cause less than 100% mortality. Now the number of deaths in each group is recorded after 24 hours and 72 hours. But in some cases of toxicity studies for some drugs the animals may not die within three days. So the period is extended up to seven to ten days. LD$_{50}$ can be estimated by different ways (Ghosh, 1994).

Litchfield and Wilcoxon graphic method
In this method different doses are given to different groups of animals and mortality recorded. Then a graph is prepared with percentage mortality vs. log dose (Litchfield, 1949).

Acute test (single dose)
Single dose of the drugs is used in animals on one occasion only, for such tests for the determination of LD$_{50}$ or median lethal dose (MLD), i.e., the dose which kills 50 percent of the animals of a particular species. LD$_{50}$ value is determined in a 24 hours' test.

Sub acute test (daily dose)
Here animals, usually rats and dogs are dosed daily starting at around expected therapeutic levels and increasing stepwise every 2 to 3 days until toxic signs are exhibited. Biochemical and hematological monitoring is carried out and blood level of the compound is checked to ensure its absorption. For a period of 2 to 3 weeks the animals are maintained at the maximum tolerated dose to allow the development of pathological symptoms and then sacrificed thereby subjected to total pathological and microscopic observations.

Sub-acute toxicity test of Tulsi Gold
Thirty male albino mice were taken and divided into three groups. The first group received control vehicle orally daily, the second group received Tulsi Gold 1 drop/daily and the last group received Tulsi Gold ½ drop/daily, for 14 days. Both the groups of mice were kept in similar laboratory conditions and were allowed to take usual food and water ad libitum. The animals were observed daily for the general conditions, gross behavior, etc. Body weights were recorded daily from the date of commencement to the experiment up to the date of their sacrifice on the 14$^{th}$ day. On the 14$^{th}$ day all the animals were sacrificed and the blood was collected by cardiac puncture to see the blood parameter.

RESULT OF TOXICITY STUDY

1. Liver Function Test

1) Determination of Alkaline Phosphates after treatment with TULSI GOLD

<table>
<thead>
<tr>
<th>Test</th>
<th>Control</th>
<th>Treated with TULSI GOLD 1 drop/daily</th>
<th>Treated with TULSI GOLD 1/2 drop/daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline Phosphates</td>
<td>355.5±4.30</td>
<td>353.7±6.30*</td>
<td>354.3±3.5</td>
</tr>
</tbody>
</table>

No. of Animals: 30

*Statistically there is no change.
2) Determination of total Bilirubin after treatment with TULSI GOLD

<table>
<thead>
<tr>
<th>Test</th>
<th>Control</th>
<th>Treated with TULSI GOLD 1 drop/daily</th>
<th>Treated with TULSI GOLD 1/2 drop/daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin (Total) mg/100ml of blood</td>
<td>1.273±0.01</td>
<td>1.261±0.04*</td>
<td>1.269±0.03</td>
</tr>
</tbody>
</table>

*Statistically there is no change.

No. of Animal: 30
3) Determination of Hb levels after treatment with TULSI GOLD

<table>
<thead>
<tr>
<th>Test</th>
<th>Control</th>
<th>Treated with TULSI GOLD 1 drop/daily</th>
<th>Treated with TULSI GOLD 1/2 drop/daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb content gm/100 ml of blood</td>
<td>15.5±0.01</td>
<td>16.2±0.06*</td>
<td>15.9±0.03</td>
</tr>
</tbody>
</table>

No. of Animals: 30

4) Determination of SGO-T & SGP-T levels after treatment with TULSI GOLD

<table>
<thead>
<tr>
<th>Test (Units/ml of serum)</th>
<th>Control</th>
<th>Treated with TULSI GOLD 1 drop/daily</th>
<th>Treated with TULSI GOLD 1/2 drop/daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGO-T</td>
<td>68±0.25</td>
<td>67.2±0.42*</td>
<td>67.6±0.32</td>
</tr>
<tr>
<td>SGP-T</td>
<td>48±0.58</td>
<td>46.8±0.09*</td>
<td>47.2±0.13*</td>
</tr>
</tbody>
</table>

No. of Animal: 30

*Statistically there is no change.
3. Kidney Function Test

**Determination of Urea levels after treatment with TULSI GOLD**

<table>
<thead>
<tr>
<th>Test</th>
<th>Control</th>
<th>Treated with TULSI GOLD 1 drop/daily</th>
<th>Treated with TULSI GOLD 1/2 drop/daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea mg/100 ml of blood</td>
<td>43.5±0.02</td>
<td>43.2±0.08*</td>
<td>43.4±0.05*</td>
</tr>
</tbody>
</table>

No. of Animals: 30

*Statistically there is no change.

![Graph showing Urea levels](image)

4. WBC count

**Determination of WBC count in thousand/dL of blood after treatment with TULSI GOLD**

<table>
<thead>
<tr>
<th>Test</th>
<th>Control</th>
<th>Treated with TULSI GOLD 1 drop/daily</th>
<th>Treated with TULSI GOLD 1/2 drop/daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC count thousands/dL of blood</td>
<td>8000± 4.12</td>
<td>7600±5.14*</td>
<td>7800±3.25*</td>
</tr>
</tbody>
</table>

No. of Animals: 30

*Statistically there is no change.

5. Body weight of Animals

<table>
<thead>
<tr>
<th>Percentage of decrease body weight in animals in respect to control</th>
<th>Treated with TULSI GOLD 1 drop/day</th>
<th>Treated with TULSI GOLD 1/2 drop/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14.04</td>
<td>5.36</td>
</tr>
</tbody>
</table>
DISCUSSION
A 14 days repeat dose (1 drop or 1/2 drop/day/mouse) oral (i.p.) toxicity test of the Tulsi gold oil extract was conducted in mice. There were no changes in signs among the test animals. Also, no differences were noted between the test and control groups in respect of food consumption, hematological, or biochemical results. There were no macroscopic or histopathological changes noted. On the divergent, all animals appeared healthy which received Tulsi Gold oil extract. Gross necropsies of all animals at the end of 2 wks revealed no hematological and biochemical changes. It is also revealed that Tulsi gold oil extract decreases body weight of animals.

ANTIBACTERIAL STUDY OF TULSI PLUS (PLATE HOLE DIFFUSION METHOD)
The plate hole diffusion assay was used to determine the growth inhibition of bacteria by plant extracts. This is also called agar diffusion test, or the Kirby-Bauer disk-diffusion method. This is a means of measuring the effect of an antimicrobial agent against bacteria grown in culture. The plate hole diffusion assay is one method for quantifying the ability of antibiotics to inhibit bacterial growth. Interpretation of results from this assay relies on model-dependent analysis, which is based on the assumption that antibiotics diffuse freely in the solid nutrient medium. In many cases, this assumption may be incorrect, which leads to significant deviations of the predicted behavior from the experiment and to inaccurate assessment of bacterial susceptibility to antibiotics. Bacteria were maintained at 4˚C on nutrient agar plates before use. Nutrient agar was prepared and 25ml of each was poured in to sterile Petri plate. The broth were inoculated with different species of bacteria and incubated at 37˚C overnight. Each Petri plate was inoculated with 0.2ml of different bacterial species mixed well, transferred in to sterile petri dishes and allows setting and this is done by spread plate method. Using a sterile cork-borer 6mm diameter, four holes per plate were made in to the set agar containing the bacterial culture. A total of 0.2ml of TULSI GOLD was poured in to the wells, the plates were kept in incubator overnight. In most studies (Naqvi et al., 1976; Leven et al., 1979; Emeruwa 1982; Singh et al., 1983), inhibition zone are compared with those obtained for antibiotics. This is useful in establishing the sensitivity of the test organism. The option condition has been established by Bauer et al. (1966), Mitscher et al. (1972).

The plate hole diffusion technique is commonly used for determination of MIC (Microbial Inhibitory Concentration) in solid media. It involves the application of antibiotic solutions of different concentrations to wells, punched into agar plates seeded with the test bacterial strain. Antibiotic diffusion from these sources into the agarose medium leads to inhibition of bacterial growth in the vicinity of the source and to the formation of clear ‘zones’ without bacterial lawn. The diameter of these zones increases with antibiotic concentration.
RESULTS

<table>
<thead>
<tr>
<th>Organism</th>
<th>Amoxicillin Con.</th>
<th>Amoxicillin Con.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>300 micro gm./ml.(5 No.)</td>
<td>200 micro gm./ml.(7 No.)</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>400 micro gm./ml.(4 No.)</td>
<td>500 micro gm./ml.(2 No.)</td>
</tr>
</tbody>
</table>

1,3,6 and 8 No holes are TULSI GOLD

DISCUSSION

Microbiological research revealed that both the g(+) and g(-) bacteria are inhibited by Tulsi gold oil extract. Antimicrobial properties of Tulsi Gold are comparable with potent antibiotic Amoxicillin.

PHOTOGRAPHS OF ANTIBACTERIAL STUDY OF TULSI PLUS (PLATE HOLE DIFFUSION METHOD)
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