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*Research Article*

# PRELIMINARY PHYSICO-CHEMICAL ANALYSIS AND PHYTO-CHEMICAL SCREENING OF TRAYODASHANGA GUGGULU

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**Abstract**

Trayodashanga guggulu is a poly herbal Ayurvedic formulation used in Ayurveda for the treatment of various inflammatory conditions associated pain and in the treatment of joint pain in various Vata vyadhis. The main ingredient is exudate of Guggulu (*Commiphora wightii* Arn.). The other drugs are Babula (*Acacia nilotica* L.), Ashwagandha (*Withania somnifera* L.), Hapusa (*Juniperus communis* L.), Guduchi (*Tinospora cordifolia* Willd.), Shatavari (*Asparagus recemosus* Willd.), Gokshura (*Tribulus terrestris* L.), Vradadaru (*Argyreia nervosa* Burm. f.), Rasana (*Pluchea lanceolata* DC.), Shatavha (*Anethum sowa* RoXb.), Sati (*Hedychium spicatum* Sm.), Yavani (*Trachyspermum ammi* L.), Sunthi (*Zingiber officinale*). The preliminary Phytochemical screening of Trayodashanga Guggulu proved the presence of Carbohydrates, Phenols, Tannins, Flavonoids, Diterpenes, Quinones and mild presence of gum and mucilage in its water extract. The physico-chemical analysis of Trayodashanga guggulu showed the Loss on drying is 3.5744%, Total ash value is 6.5717%, Acid insoluble ash is 2.4180%, Water soluble ash is 2.0748%, Water soluble extraction is 25.0029%, Alcohol soluble extraction is 3.2449%. Here in the present study an attempt was made to analyse the formulation Trayodashanga Guggulu phyto-chemically and physico-chemically.

**Key words:** Trayodashanga guggulu; Phyto-chemical, Physico-chemical; Inflammation; Pain.

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## INTRODUCTION

Trayodashanga guggulu is a compound Ayurvedic polyherbal formulation commonly used for reducing pain and inflammation in many diseases like low back ache, muscle stiffness, jaw stiffness, lumbar spondylosis, low back stiffness etc. This compound Ayurvedic formulation contains 13 herbal drugs. The main ingredient is exudate of Guggulu (*Commiphora wightii* Arn.), a small tree belonging to the family Burseraceae and is used after shodhana (purification). The other drugs are Babula (*Acacia nilotica* L.), Ashwagandha *Withania somnifera* (L.), Hapusa *Juniperus communis* L.), Guduchi (*Tinospora cordifolia* Willd.), Shatavari (*Asparagus recemosus* Willd.), Gokshura (*Tribulus terrestris* L.), Vradadaru (*Argyreia nervosa* Burm. f.), Rasana (*Pluchea lanceolata* DC.), Shatavha (*Anethum sowa* Roxb.), Sati (*Hedychium spicatum* Sm.), Yavani (*Trachyspermum ammi* L.), Sunthi (*Zingiber officinale*). Ayurvedic Formulary of India,[1] Ayurvedic Pharmacopoeia of India,[2] and Bhaishaja Ratnavali[3] explains the formulation, its pharmaceutical method of preparation and its use in treatment of joints related problems, stiffness, inflammation, arthritis, stiffness of jaw, ligament injury and fractures. (Table 1) Here in the present study an attempt was made to prepare the drug by classical method and to analyse its phyto-chemical and physico-chemical parameters. The preliminary study was done in The Tamil Nadu Dr. M.G.R. Medical University, Chennai.

## METHODOLOGY

All the drugs were pharmacognostically identified for its genuinity. The formulation Trayodashanga guggulu was prepared as per the classical method. After doing shodhana of guggulu as per the classical method the fine powder of remaining 13 drugs were added and pounded thoroughly to get a homogenous mixture by adding a little quantity of ghee.

## PRELIMINARY PHYTOCHEMICAL SCREENING OF TRAYODASHANGA GUGGULU

The preliminary phytochemical screening test was carried out for each extracts of Trayodashanga guggulu as per the standard procedure mentioned here under.

### Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

#### *Mayer's Test*

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colour precipitate indicates the presence of alkaloids.

#### *Dragendroff's Test*

Filtrates were treated with Dragendroff's reagent (Potassium Bismuth Iodide). Formation of a red precipitate indicates the presence of alkaloids.

#### *Wagner's Test*

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

### Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were usedto test for the presence of carbohydrates.

#### *Molisch's Test*

To 2 ml of plant sample extract, two drops of alcoholic solution of α-naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

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***Benedict's Test***

Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

**Detection of saponins**

***Foam Test***

0.5 g of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

**Detection of phenols**

***Ferric Chloride Test***

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

**Detection of tannins - Gelatin Test**

The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

**Detection of Flavonoids**

***Alkaline Reagent Test***

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

***Lead acetate Test***

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

**Detection of diterpenes - Copper Acetate Test**

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

**Test for Quinones**

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

**Gum and Mucilage**

To 1 ml of extract add 2.5 ml of absolute alcohol and stirring constantly. Then the precipitate was dried in air and examine for its swelling properties. Swelling was observed that will indicate presence of gum and mucilage. The Preliminary phytochemical studies of aqueous extract of Trayodashanga Guggulu were done using standard procedures. The results were presented in tables. The present study reveals that the bioactive compounds were present in all the extracts of Trayodasanga Guggulu

**PHYSICOCHEMICAL ANALYSIS OF TRAYODASHANGA GUGGULU**

The preliminary physicochemical screening test was carried out for Trayodasanga Guggulu as per the standard procedures mentioned hereunder.

**Loss on Drying**

An accurately weighed 1g of Trayodasanga Guggulu formulation was taken in a tarred glass bottle. The crude drug was heated at $105^0$C for 6 hours in an oven till a constant weight. The Percentage moisture content of the sample was calculated with reference to the shade dried material.

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**Determination of total ash**

Weighed accurately 2 g of Trayodasanga Guggulu formulation was added in crucible at a temperature 600$^0$C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

**Determination of acid insoluble ash**

Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble as was calculated with reference to the air dried drug.

**Determination of water soluble ash**

Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15 min at a temperature not exceeding 450$^0$C in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

**Determination of water soluble Extractive**

5gm of air dried drug, coarsely powered Trayodasanga Guggulu was macerated with 100ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The Solution was filtered and 25 ml of filtrated was evaporated in a tarred flat bottom shallow dish, further dried at 100$^0$C and weighted. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

**Determination of alcohol soluble extractive**

1 gm of air dried drug coarsely powdered Trayodasanga Guggulu was macerated with 20 ml alcohol in closed flask for 24 hrs. With frequent shaking, it was filtered rapidly taking precaution against loss of alcohol 10ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100$^0$C and weighted. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

## RESULTS AND OBSERVATION

The preliminary Phytochemical screening of Trayodashanga Guggulu proved the presence of Carbohydrates, Phenols, Tannins, Flavonoids, Diterpenes, Quinones and mild presence of gum and mucilage in its water extract. (Table 2)

The physico-chemical analysis of Trayodashanga guggulu showed the Loss on drying is 3.5744%, Total ash value is 6.5717%, Acid insoluble ash is 2.4180%, Water soluble ash is 2.0748%, Water soluble extraction is 25.0029%, Alcohol soluble extraction is 3.2449%. (Table 3)

## DISCUSSION

Tryodashanga guggulu contains Aabha, Ashwagandha, Hapusha, Guduchi, Shatavari, Gokshura, Vriddhadaru, Rasna, Shatapushpa, Karchur, Yavani, Shunthi, Guggulu (shoditha) and Ghee. In the combination of Tryodashanga guggulu, concentration of Guggulu is found to more, which has the pravabha of Vatahara (Normalizing the Vata dosha). In this formulation, all 10 Dravyas (drugs) have dominant Tikta rasa (bitter), 6 Dravyas, have dominant Kashaya Rasa (astringent taste), 6 Dravyas having dominancy of Katu rasa (pungent taste), and 4 dravyas having dominant of Madhura rasa (sweet taste).

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**Table 1: Ingredients of Trayodashanga Guggulu and the properties of the drugs**

| Ingredient | Botanical Name | Rasa | Guna | Virya | Vipaka |
|---|---|---|---|---|---|
| Abha/Baboola | *Acacia arabica* | Kashaya | Guru, Ruksa | Ushna | Katu |
| Aswagandha | *Withania somnifera* | Tikta, Kashaya | Laghu, Snigdha | Ushna | Madhura |
| Hapusha | *Juniperus communis* | Tikta, Katu | Laghu, Ruksa | Ushna | Katu |
| Guduchi | *Tinospora cordifolia* | Tikta, Kashaya | Guru, Snigdha | Ushna | Madhura |
| Satavari | *Asaparagus racemosus* | Madhura, Tikta | Guru, Snigdha | Sita | Madhura |
| Gokshura | *Tribulus terrestris* | Madhura | Guru, Snigdha | Ushna | Madhura |
| Vrddhadaru | *Argyeria speciosa* | Katu, Tikta, Kashaya | Laghu, Snigdha | Ushna | Madhura |
| Nagara | *Zingiber officinalis* | Katu | Laghu, Snigdha | Ushna | Madhura |
| Rasna | *Pluchea lanceolata* | Tiktaa | Gurua | Ushna | Katu |
| Sathawa | *Anethum sowa* | Katu, Tikta | Laghu, Tiksna, Snigdha | Ushna | Katu |
| Yavani | *Trachyspermum ammi Linn* | Katu, Tikta | Laghu, Ruksa, Tiksna | Ushna | Katu |
| Shati | *Hedychium spicatum* | Katu, Tikta, Kashaya | Laghu, Ruksa, Tiksna | Ushna | Katu |
| Guggulu | *Commiphora wightii* (Arn.) | Tikta, Katu | Laghu, Ruksha, Tikshna, Sukshma, Sara | Ushna | Katu |

**Table 2: Phytochem]=ical screening of trayo dashanga guggulu**

| S.No. | Phytochemicals | Test Name | $H_2O$ Extract |
|---|---|---|---|
| 1 | Alkaloids | Mayer's Test | -ve |
| | | Dragendroff's Test | -ve |
| | | Wagner Test | -ve |
| 2 | Carbohydrates | Molisch's Test | +ve |
| | | Benedict Test | +ve |
| 3 | Saponin | Foam Test | -ve |
| 4 | Phenols | Ferric Chloride Test | +ve |
| 5 | Tannins | Gelatin Test | +ve |
| 6 | Flavonoids | Alkaline Reagent Test | -ve |
| | | Lead acetate | +ve |
| 7 | Diterpenes | Copper Acetate Test | +ve |
| 8 | Quinones | Test for Quinones | +ve |
| 9 | Gum & Mucilage | Test for Gum & Mucilage | +ve (Mild) |

**+ve/-ve present or absent if component tested**

**Table 3: Physicochemical analysis of trayo dashanga guggulu**

| S.No | Parameters | Percentage |
|---|---|---|
| 1 | Loss on drying | 3.5744% |
| 2 | Total ash value | 6.5717% |
| 3 | Acid insoluble ash | 2.4180% |
| 4 | Water soluble ash | 2.0748% |
| 5 | Water soluble extraction | 25.0029% |
| 6 | Alcohol soluble extraction | 3.2449% |

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Among these three, Katu Rasa has potential of Agni sandipana (stimulates Agni or digestive fire) & Mamsavilekhanam, which helps to normalize the digestive fire to form nutritional Anna Rasa (essence of food) as substrate which further give qualitative nutrition to succeeding Dhatus (tissues) & help in modification or normalization of Dhatwagni. It also helps to pacify Vata in the affected sandhi (joint). As Dhatwagni increases, nutrition of all dhatus will be increased then asthi and majja dathu may be get stable and asthi dhatu and majja dhatu kshaya will be decreased. So degeneration in asthi dhatu may not occur rapidly. In Trayodashanga Guggulu out of 13 dravyas 10 have dominant tikta rasa and also it contains ghrita. Tikta Rasa is having deepana, pachana and rochana properties. So it helps in the improvement of the general condition of health and thus strengthens the body as well as joints. Kashaya Rasa also has property of Sharira-Kleda Shoshana. All these dominant Rasa in this formulation thus helps in breakage of pathogenesis of Disease. Besides this, there is dominancy of Laghu, Ruksha & Tikshna Gunas in the Tryodashanga Guggulu which also helps in Sama dosa and Vata shamana.The formulation of Tryodashanga guggulu has 10 Dravyas with dominance of Ushnavirya which also helps to pacify the Vata Dosha. With all these properties, Sukshma property of Guggulu helps in Bhedana of Avarana, Vatanulomana, Vatasamana. Screening the analytical study it could be said the formulation contains Carbohydrates, Phenols, Tannins, Flavonoids, Diterpenes, Quinones and mild presence of gum and mucilage in its water extract.

## CONCLUSION

Tryodashanga guggulu is a excellent formulation for joint disorders etc. The present study is only a preliminary study. Yet more studies should be conducted to prove scientifically The preliminary Phytochemical screening of Trayodashanga Guggulu proved the presence of Carbohydrates, Phenols, Tannins, Flavonoids, Diterpenes, Quinones and mild presence of gum and mucilage in its water extract. The physico chemical analysis of Trayodashanga guggulu showed the Loss on drying is 3.5744%, Total ash value is 6.5717%, Acid insoluble ash is 2.4180%, Water soluble ash is 2.0748%, Water soluble extraction is 25.0029%, Alcohol soluble extraction is 3.2449%. Water soluble extraction is more in the study. This gives an idea if this formulation is given along with any kwatha (decoction) the action will be still more fast and effective.

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