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## Research Article

# PRELIMINARY PHYTOCHEMICAL ANALYSIS OF LEAVES OF *GUCHAKARANJA* (*QUASSIA INDICA* GAERTN)

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**ABSTRACT**

The drug *Guchakaranja* is mentioned as one of *Karanja* variety by *Raj Nighantu* and *Nighantu Ratnakara*. The references of the drug are not seen in other classical textbooks of Ayurveda and the only references seen in these two *Nighantus*. The drug had been correlated to *Karinjotta*, a locally available plant in Kerala. The drug *Guchakaranja* is botanically correlated as *Quassia indica* Gaertn (*Samadera indica* Gaertn) belonging to the family Simaroubaceae commonly known as Niepa bark tree. The drug had been extensively used in folklore practices and usage of the plant in main stream clinical practices is less. So giving a standardization and to justify its traditional usages preliminary phyochemical analysis had been done. The preliminary phytochemical analysis aims at analyzing the physico chemical property of drugs, their qualitative analysis, ash values, extractive values, moisture and volatile contents, estimation of Tannins and Phenols and HPTLC. Previous studies are available regarding the Qualitative Analysis of phytochemicals, tannin and total phenolic estimation. References regarding ash values, quantitative estimation of fiber, reducing sugar and total sugar, pH, cold and hot alcohol and water soluble extractives, moisture content were not available from previous research works On analyzing the phytochemical constituents present in the crude drug, the drug revealed the presence of alkaloids, flavonoids, saponins, carbohydrates, proteins, phenols, steroids, and tannins. This phytoconstituents present in the drug may be responsible for specific action of the drug. This preliminary phytochemical evaluation may be helpful to identify the potential of *Guchakaranja* and should be helpful in developing new formulations with additional therapeutic effect.

**KEYWORDS:** *Guchakaranja*, *Quassia indica* Gaertn, Phytochemical evaluation.

## INTRODUCTION

Ayurvedic health care system mainly based on plant and plant based products. Plants have been the primary basis for drug discoveries and developing new drugs. Along with the conservation and cultivations of medicinal plants it is also mandatory to explore the large number of *Anukta dravya* which are still waiting to move towards main stream clinical practice of Ayurveda as well as to enrich the Ayurvedic Pharmacopeia of India.

Some *Dravyas* which are mentioned in *Ayurvedic Nighantus* are still unknown and there are not using the main stream clinical practices. Also folklore system of medicine is a rich source of knowledge which gives excellent unfailing remedies for a number of clinical conditions. So we have to explore the identity and utility of the drugs mentioned in *Nighantus* by searching its folklore and traditional uses.

The present study drug *Guchakaranja* is botanically correlated as *Quassia indica* Gaertn (*Samadera indica* Gaertn) belonging to the family Simaroubaceae commonly known as Niepa bark tree. The Ayurvedic reference of the drug can be seen in *Raja Nighantu*[1] and *Nighantu Ratnakara*[2], in these *Nighantus* the drug is indicated for *Vata rogas*, *Visha, Kandu, Kushta, Vicharchika* and *Twak doshas*. The reference from *Hortus Malabaricus*[3] text covering the medicinal drugs available and practiced in Kerala in the 17th century, revealed its usage in inflammatory conditions, fever, skin diseases and rheumatic complaints. The drug is popularly known as '*Karinjotta'* in Kerala. Recent ethno pharmacological studies showed *Quassia indica* Gaertn is used in many parts of world as febrifuge, tonic, stomachic, emmenagogue and for the treatment of many skin diseases. Leaves are used to cure cough, erysipelas and for killing head lice. Research works have proved its various pharmacological activities like anti inflammatory, anti oxidant, anti spasmodic, anti microbial, anti

helminthic, antiviral, anti feedant and growth regulating.[4-5]

Till now classical Ayurvedic preparations of the drug has not been the subject of any Ayurvedic research. For analyzing the genuinity and purity of the drug phytochemical evaluation of the drug has been conducted. The preliminary phytochemical analysis aims at analyzing the physico chemical property of drugs, their qualitative analysis, ash values, extractive values, moisture and volatile contents, estimation of Tannins and Phenols and HPTLC.

## MATERIALS AND METHODS

### Materials of Phytochemical analysis

### Collection of the plants

The fresh leaves of *Guchakaranja (Quassia indica* Gaertn) were collected from the Puthiyakavu locality of Tripunithura. The sample was identified as genuine by the Pharmacognostic studies, conducted in the department of *Dravyaguna Vijnanam*, Government Ayurveda College, Tripunithura, Kerala. The leaves were washed with water thoroughly to remove physical impurities like soil, mud etc., and then chopped and dried well in sun. It was then powdered and kept in airtight containers. The Phytochemical analysis was done at Drug standardization unit of Department of *Dravyaguna Vijnanam*, Government Ayurveda College, Tripunithura, Kerala.

**Fig 1: *Quassia indica* Gaertn**

**Fig 2: Leaves of *Quassia indica* Gaertn**

### Preliminary Physical and Phytochemical Evaluation

Preliminary phytochemical analysis was carried out to identify the secondary metabolities present in the drug. The physical and preliminary phytochemical analysis was done by standard procedures mentioned in the Ayurvedic Pharmacopoeia of India.[6] Physical evaluation includes Foreign matter, Total ash, Acid Insoluble Ash, Water Insoluble Ash, Moisture Content, Volatile oil, Fiber, Tannin Content, Total sugar, Reducing sugar, Phenol and pH. Qualitative analysis was done to analyze the presence of steroid, flavonoid, phenol, alkaloid, tannin, carbohydrate, proteins and saponin. Extractive values include water soluble and alcohol soluble extractives and successive solvent extraction. The extractive values represent the percentage of organic constituents present in the drug. Water soluble extractives represent the organic constituents such as glycosides, tannins and mucilage and alcohol soluble extractives represent the presence of alkaloids, flavonoids, phenols, sugar etc. HPTLC was done to analyze the presence of chemical constituents in the drug.

### Preliminary Qualitative Analysis

#### 1. Alkaloids

#### a) Dragendroff's test

To 0.5ml of alcoholic extract of powder of leaves of *Guchakaranja (Quassia indica* Gaertn) was taken in test tube, 2.0ml of Hydrochloric acid solution was added. To this acidic medium, 1.0ml of Dragendroff''s reagent was added. An orange-red precipitate produced immediately indicated the presence of alkaloid.

#### b) Meyer's test

To 10ml of the solution of alcoholic extracts of powder of leaves of *Guchakaranja (Quassia indica* Gaertn) was taken in test tubes, a few drops of Meyer's reagent was added. Formation of white or pale precipitate indicated the presence of alkaloid.

#### 2. Flavonoids

To 0.5ml of the solution of alcoholic extract of powder of leaves of *Guchakaranja (Quassia indica* Gaertn) was taken in test tubes, 5 -10 drops of Dilute hydrochloric acid was added and a small piece of magnesium were added and the solution was boiled for few minutes. Presence of pink colour indicated Flavonoids.

#### 3. Saponins

To 5ml of the solution of aqueous extract of powder of leaves of *Guchakaranja (Quassia indica* Gaertn) was taken in test tube, 1 -3 drops of sodium bicarbonate solution was added. The mixture was shaken vigorously and kept for 3 minutes. A honey

comb like froth formation in test tube indicated the presence of Saponins.

**4. Carbohydrates**

**a) Fehling's test**

To 2ml of aqueous extract of powder of leaves of *Guchakaranja (Quassia indica* Gaertn) was taken in test tube, a mixture of equal parts of Fehling's solution A and B were added. The test tube was then boiled for few minutes. Formation of red or brick precipitate indicated the presence of carbohydrates.

**b) Benedict's test**

To 0.5ml of aqueous extract of powder of leaves of *Guchakaranja (Quassia indica* Gaertn) was taken in test tube; 5ml of Benedict's reagent was added and boiled for 5minutes. Formation of bluish green colour in test tube indicated the presence of carbohydrates.

**5. Proteins**

**a) Ninhydrin test**

To 1ml of the solution of aqueous extract of powder of leaves of *Guchakaranja (Quassia indica* Gaertn) as taken in test tube, 5ml of Ninhydrin solution was added and heated in a boiling water bath for 2-3 minutes. Formation of blue or purple colour indicated the presence of proteins.

**6. Phenols**

**a) Ferric Chloride test**

To 1.0ml of the solution of the alcoholic extract of powder leaves of *Guchakaranja (Quassia indica* Gaertn) was taken in test tubes, 2.0ml of distilled water was added followed by addition of a few drops of 10% aqueous ferric chloride solution. Formation of blue or green colour indicated the presence of phenols.

**b) Lead acetate test**

1.0ml of the solution of the alcoholic extracts of powder of leaves of *Guchakaranja (Quassia indica* Gaertn) was taken in test tube. 5ml distilled water was added followed by few drops of 1% aqueous solution of lead acetate. The formation of yellow precipitate in test tubes indicated the presence of phenols.

**7. Steroids**

To 2.0ml of the solution of chloroform extracts of powder of leaves of *Guchakaranja ( Quassia indica* Gaertn) was taken in test tube, 1.0ml of concentrated Sulphuric acid was added carefully along the sides of the test tube. A red colour was produced in the chloroform layer indicated the presence of steroids.

**8. Tannins**

**a) Ferric chloride test**

To 1-2ml of the solution of aqueous extract of powder of leaves of *Guchakaranja (Quassia indica* Gaertn) was taken in test tubes, a few drops of 5% aqueous ferric chloride solution was added. A bluish black colour formed which disappeared on addition of diluted Sulphuric acid, forming a yellow brown precipitate indicated the presence of tannins.

**b) Lead acetate test**

To 5ml of the solution of the aqueous extracts of powder of leaves of *Guchakaranja (Quassia indica* Gaertn) was taken in separate test tubes, few drops of 1% solution of lead acetate was added. Formation of yellow or red precipitate indicated the presence of tannin.

The other procedures of moisture content, Volatile oil extraction, estimation of sugar content, tannin, phenols, extractive values had been done as per standard procedures mentioned in Ayurvedic Pharmacoepia of India.

## RESULTS

Results of various phytochemical analysis are tabulated below:

**Table 1: Results of physico-chemical parameters**

| S.No. | Experiments | *Guchakaranja* |
|---|---|---|
| 1 | Foreign matter | Nil |
| 2 | Total ash | 8.2% |
| 3 | Acid Insoluble Ash | 4.45 % |
| 4 | Water Insoluble Ash | 8.6% |
| 5 | Moisture Content | 24.5% |
| 6 | Volatile oil | 15% |
| 7 | Fiber | 59.5% |
| 8 | Tannin Content | 44.625% |
| 9 | Total sugar | Trace amount |
| 10 | Reducing sugar | Trace amount |
| 11 | Phenol | 16.05µg/g |
| 12 | pH | 6.78 |

**Table 2: Results of alcohol and water extractive values**

| S.No. | Type of Extractives | *Guchakaranja* |
|---|---|---|
| 1 | Cold Alcohol soluble | 5.1% |
| 2 | Hot Alcohol soluble | 9.5 % |
| 3 | Cold water soluble | 13.7% |
| 4 | Hot water soluble | 3.9% |

**Table 3: Results of successive solvent extraction**

| S.No. | Solvent | % of extractive values of *Guchakaranja* |
|---|---|---|
| 1 | Petroleum ether | 4.29% |
| 2 | Cyclohexane | 2.18 % |
| 3 | Acetone | 3.98 % |
| 4 | Alcohol | 3.65% |

**Table 4: Results of qualitative analysis of crude drug**

| Experiment | *Guchakaranja* |
|---|---|
| 1) Alkaloids | |
| a) Dragendroff"s test | + |
| b) Meyer's test | - |
| 2) Flavonoids | - |
| 3) Saponins | ++ |
| 4) Carbohydrates | |
| a) Fehling's test | ++ |
| b) Benedict's test | ++ |
| 5) Proteins | - |
| 6) Phenols | |
| a) Ferric chloride test | + |
| b) Lead acetate test | + |
| 7) Steroids | + |
| 8) Tannins | |
| a) Ferric chloride test | + |
| b) Lead acetate test | + |

**Table 5: Results of qualitative analysis of extractives of *Guchakaranja***

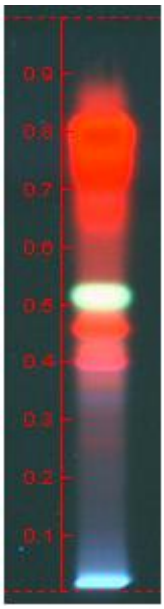
| S.No. | Extract | Steroids | Alkaloids | Flavonoids | Phenols |
|---|---|---|---|---|---|
| 1 | Petroleum ether | + | + | + | + |
| 2 | Cyclohexane | + | + | - | - |
| 3 | Acetone | - | - | - | - |
| 4 | Alcohol | + | + | + | - |

**Table 6: Results of qualitative analysis of ash of *Guchakaranja***

| S.No. | Experiment | *Guchakaranja* |
|---|---|---|
| **Acid radicals** | | |
| 1 | Carbonate | - |
| 2 | Phosphate | + |
| 3 | Chloride | + |
| 4 | Sulphate | + |
| **Basic radicals** | | |
| 5 | Pottasium | - |

**High performance thin layer chromatography (HPTLC)**

High performance thin layer chromatography (HPTLC**)** was done using the methanolic extract of leaves of *Quassia indica* Gaertn having mobile phase as Toluene: Ethyl acetate: Formic acid: Methanol (7:5:1:0.5). The development of the plate is done in the CAMAG 10×10cm Twin trough chamber and visualized under UV at 254nm and 366nm after derivatization using 10% sulphuric acid reagent.



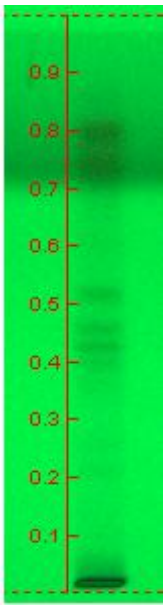


**Fig 3: HPTLC Plate at 366nm** **Fig 4 :HPTLC Plate at 254nm**

**Rf Value & % Area of *Karinjotta* leaf at 254nm**

**Table 7: Rf Value & % Area of *Karinjotta* leaf at 254nm**

| Peak No | Rf Value | Area (AU) | % Area (AU) |
|---|---|---|---|
| 1 | 0.14 | 175.9 | 1.04 |
| 2 | 0.21 | 604.0 | 3.57 |
| 3 | 0.26 | 551.1 | 3.26 |
| 4 | 0.37 | 287.9 | 1.70 |
| 5 | 0.40 | 392.6 | 2.32 |
| 6 | 0.43 | 2255.4 | 13.34 |
| 7 | 0.46 | 345.8 | 2.05 |
| 8 | 0.52 | 3583.9 | 21.19 |
| 9 | 0.61 | 405.1 | 2.40 |
| 10 | 0.75 | 5671.0 | 33.54 |
| 11 | 0.80 | 2636.7 | 15.59 |

Total peak no – 11; Total area – 16909.4 (au**)**

**Table 8: Rf Value & % Area of *Karinjotta* Leaf at 366nm**

| Peak No | Rf Value | Area (AU) | % Area (AU) |
|---|---|---|---|
| 1 | 0.05 | 1241.8 | 4.55 |
| 2 | 0.21 | 342.5 | 1.25 |
| 3 | 0.26 | 412.0 | 1.51 |
| 4 | 0.40 | 3274.9 | 11.99 |
| 5 | 0.56 | 2567.2 | 9.40 |
| 6 | 0.52 | 7750.8 | 28.38 |
| 7 | 0.76 | 2330.4 | 8.53 |
| 8 | 0.80 | 8944.0 | 32.75 |
| 9 | 0.86 | 446.8 | 1.64 |

Total peak no – 09: Total area –27310.4 (au)

## DISCUSSION

The detailed phytochemical analysis was carried out to determine the quality and purity of the drug. These studies showed presence of major active ingredients which are responsible for specific pharmacological actions. On analyzing the phytochemical constituents present in the crude drug, the drug revealed the presence of alkaloids, flavonoids, saponins, carbohydrates, phenols, steroids, and tannins. These secondary metabolites are reported to have many biological and therapeutic actions. The extraction yield calculated for petroleum ether, cyclohexane, acetone and alcohol, the petroleum ether extract showed highest percentage of yield. On the HPTLC analysis of the drug *Quassia indica* Gaertn revealed a total peaks of 11 with total area of 16909.4 (au) at 254 nm. At 366nm a total of 9 peaks were obtained with total area of 27310.4 (au). The flavonoids, saponins and fibre content in the drug *Quassia indica* Gaertn may be responsible for its anti-inflammatory and analgesic properties. Thus the phytochemical analysis of the leaves of *Guchakaranja (Quassia indica* Gaertn) justifies its use in traditional practices for various ailments. More research regarding isolation of more phytochemicals and pharmacology study on this herbal plant is still essential so as to explore the plant regarding its medicinal importance.

## CONCLUSION

The drug *Guchakaranja (Quassia indica* Gaertn) has been widely used in traditional practices in various diseased conditions. For giving a validation to traditional practices and to standardize the drug the preliminary phytochemical analysis of the drug had been carried out. The phytochemical evaluation showed presence of the phytoconstituents that may be responsible for specific therapeutic action of the drug.

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