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QUALITATIVE AND QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS ON SINGLE DRUG NARUVILI CHOORANAM (*CORDIA DICHOTOMA* FORST F.)

Dr. Kalaipriya R.[1]*, Subash Chandran G.[2] and Komalavalli Alias Mehala T.[3]

[1]PG Scholar, Department of Pothu Maruthuvam, Government Siddha Medical College and Hospital, Palyamkottai, Tirunelveli.
[2]Associate Professor, Department of Pothu Maruthuvam, Government Siddha Medical College.
[3]Professor and HOD, Department of Pothu Maruthuvam, Government Siddha Medical College and Hospital, Palyamkottai, Tirunelveli.

***Corresponding Author: Dr. Kalaipriya R.**

PG Scholar, Department of Pothu Maruthuvam, Government Siddha Medical College and Hospital, Palyamkottai, Tirunelveli.

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ABSTRACT

Background: Siddha medicine is a indigneous traditional system of medical practice that emerged in South India and is recognized as one of the India's ancient forms of medicine. *Cordia dichotoma* G.Forst is a small to medium size deciduous tree with a short crooked trunk, short bole and spreading crown. It belongs to the family Boranginaceae. The medicine Naruvili chooranam (*Cordia dichotoma* G Forst) is a single drug preparation which contains leaves of *Cordia dichotoma* indicated in Siddha textbook in the management of Iraippu Noi (Bronchial Asthma**). Aim and Objectives**: An aim of the study is to evaluate the qualitative and quantitative analysis of phytochemicals in Naruvili chooranam. **Methodology:** The chooranam was prepared as mentioned in Siddha literatures and used for experimental purpose. **Results and conclusion:**Preliminary phytochemical analysis shows there was a presence of Saponins, Tannins, Terpenoids, Alkaloids, Flavanoids and Glycosides and further quantitative analysis showed each 100 mg of Naruvili chooranam contains 144mcg of Saponin, 27mcg of Tannin, 5mcg of Terpenoids, 98mcg of Alkaloids, 104mcg of Flavanoids, 33mcg of Glycoside. This study serves as a benchmark for creating a standardized pharmaceutical product and conducting quality control research in future.

KEYWORDS: Bronchial Asthma, Naruvili chooranam, Iraippu Noi, Cordia dichotoma.

INTRODUCTION

For the countless years, the Siddhars possessed extensive knowledge about the healing properties of plants and animals in the world. Their wealth of information on the medicinal uses of these herbs has been documented over centuries in manuscripts. Siddha medicine offers a comprehensive approach to healthcare, including prevention, promotion, cure, rejuvenation, rehabilitation through a scientific and holistic methodology. *Cordia dichotoma G.Forst* (Naruvili) is a small to medium size deciduous tree with a short crooked trunk, short bole and spreading crown. It belongs to the family Boranginaceae. Leaves are simple, entire and slightly dentate, elliptical-lanceolate to broad ovate with a round and cordate base. According to T.V. Sambasivam Pillai dictionary, it literally known as Fragrant tree. Naruvili is one of the traditionally important medicinal plant available all over the India. Polyherbal formulations are extensively used by the masses in India for the treatment for the common cold, catarrh, cough, respiratory distress, fever of which Naruvili is chief ingredient. Phyochemical analysis includes both qualitative and quantitative assessments. Qualitative analysis focuses on determining the presence or absence of specific phytochemicals, while quantitative analysis determines the amount or concentration of phytochemicals in the plant sample.

Kingdom: Plantae
Division: Magnoliophyta
Subclass: Astaridae
Order: Lamiales
Family: Boranginaceae
Genus: Cordia
Species*: C.dichotoma* Forst.f
Fragrant: Manjack.

VERNACULAR NAMES

English: Sebestem plum, soap berry
Tamil : Kalvirusu, Vidi, Naruvili
Telugu: Botuku, Nakkaeru
Malayalam: Naruviri
Sanskrit: Sleshmataka, Shelu, Bahuvarka
Arabic: Dibaka
Hindi: Lasora

Kannada: Mannadike
Gujarati: Vadgundo, Gundo
Nepali :Bohori, Kalobohori
Thai : Mandong, Manma, Phakmong.
Japanese : Kendel
Sumatran : Nunang.

Fig. 1: Cordia dichotoma (Naruvili).

MATERIALS AND METHODS

Naruvili leaves were collected around the areas of Nagercoil and it was authenticated by the faculties at Department of Gunapadam, GSMC Palayamkottai. It was dried in the shade, ground into coarse powder, filtered to obtain chooranam and then stored in an airtight container labelled as Naruvili Chooranam for experimental use. Qualitative analysis was conducted at Siddha Regional Research Institute, Trivandrum, Kerala. Further quantitative analysis was conducted at Inbiotics institute, Nagercoil, Kanyakumari district, Tamil Nadu.

II. Preliminary Phytochemical Analysis

1. Test for Saponins

To a few mg of extract distilled water is added and shaken well. The formation of foam indicates the presence of saponin.

2. Test for Tannin

To Substance in water is added with 5 % alcoholic ferric chloride. Dark blue colour shows presence of tannin.

3. Test for Terpenoids

To a few mg of extract in chloroform, add conc. H_2SO_4. Presence of dark brown precipitate indicates the presence of terpenoids.

4. Test for Phenol

To Substance in water is added with 5 % alcoholic ferric chloride. Dark blue or green colour shows presence of phenol.

5. Test for Steroids (Lieberman Burchard Test)

To a few mg of the extract 2 ml of chloroform is added in a dry test tube. Few drops of acetic acid is added, heated and few drops of acetic anhydride and 2 drops of concentrated sulphuric acid are added. The green colour indicates the presence of steroid.

6. Test for Quinones

To a few mg of extract, add few drops of concentrated sulphuric acid. Appearance of red colour shows the presence of quinone.

7. Test for Glycosides

Substance is treated with anthrone and concentrated sulphuric acid. On heating over a water bath, the appearance of green colour shows the presence of glycoside.

8. Test for Carbohydrates

To the sample solution, added few drops of α-naphthol and 2-3 ml conc. H_2SO_4. The appearance of reddish violet or purple ring at the junction of two liquids indicates the presence of Carbohydrates.

9. Test for Alkaloids (Dragendorff's Test)

Few mg of extract in separate test tube was warmed with 2% Sulphuric acid for 2 minutes. And it was filtered in separate test tube and few drops of Dragendorff's reagent were added. The presence of orange red precipitates indicates the presence of alkaloids.

10. Test for Flavonoid

To the substance in alcohol add 10% NaOH or ammonia. A dark yellow colour indicates the presence of flavonoid.

11. Test for Proteins (Biuret test)

To the sample solution in a test tube, add sodium hydroxide solution and then add a few drops of very dilute (1 %) copper II sulphate solution and mix gently. Appearance of purple colour indicates the presence of protein.

METHODOLOGY

PHYTOCHEMICAL QUANTITATIVE ANALYSIS

Quantitative Estimation of flavanoids

Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume).

After 5 min 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The

absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g dried extract).

Quantitative Estimation of Saponins

Take 1ml of sample add 2ml of 8 % Vanilin in ethanol was added, mixed well and the 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 60^0c for 10min. After 10 minutes the tubes were cooled. Absorbance was measured at 544nm against reagent blank. Diosgeninis used as a standard material and compared the assay with Diosgenin equivalents. Results were expressed as Diosgenin equivalents (mg catechin/g dried extract).

Quantitative Estimation of Tannins

The tannin contents or Proanthocyanidin were determined by method of Broadhurst et al., 1978 with slight modification, using tannic acid as a reference compound. One milliliter of the extract was mixed with 5ml of vanillin hydrochloride reagent (mix equal volumes of 8% HCL in methanol and 4% vanillin in methanol). The mixed was allowed to stand for 20mins and measure the absorbance at 500nm. The standard graph was plotted for working standard tannic acid solution (20 to 200µg/µl).

Quantitative Estimation of Phenolic Compounds

The total phenolics content in different solvent extracts was determined with the Folin- Ciocalteu's reagent (FCR)**.** In the procedure, different concentrations of the 1 ml of the extract were mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 min 4 ml of 7% sodium carbonate solution was added. The final volume of the tubes were made upto 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using Gallic acid solutions as standard (20 to 200µg/µl).

Quantitative Estimation of Glycoside

Take 10ml of the extract and 10ml of Baljet's reagent (95ml 1% picric acid+ 5ml of 10 % aqueous sodium hydroxide) are taken and allowed to stand for one hour. Then dilute the solution with 20ml distilled water and mix. Read the intensity of the colour obtained against blank at 495nm using a spectrophotometer. The difference between test and control is taken for calculation. Standard graph can be prepared using standard digitoxin.

Quantitative Estimation of Alkaloids

To 1ml of Methanolic extract add 5 ml pH 4.7 phosphate Buffer and 5 ml BCG solution then shake the mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalent.

Quantitative Estimation of Terpenoid

Total terpenoid content was determined by the method of Ghorai et al (2012) 17. To 1 mL of the plant extract, 3 mL of chloroform was added. The sample mixture was thoroughly vortexed and left for 3 min and then 200 µl of concentrated sulfuric acid (H2SO4) was added. Then it was incubated at room temperature for 1.5h-2h in dark condition and during incubation a reddish brown precipitate was formed. Then carefully and gently, all supernatant of reaction mixture was decanted without disturbing the precipitation. 3 mL of 95% (v/v) methanol was added and vortexed thoroughly until all the precipitation dissolve in methanol completely. The absorbance was read at 538 nm using UV/visible spectrophotometer.

RESULTS

PRELIMINARY QUALITATIVE ANALYSIS OF PHYTOCHEMICALS

TABLE 1.

Tests	Result
Saponins	+
Tannins	+
Phenols	-
Terpenoids	+
Alkaloids	+
Flavanoids	+
Steroids	-
Glycosides	+
Carbohydrates	-
Quinones	-
Proteins	-

+ present
- absent

DISCUSSION

Phytochemicals are plant based derivatives of bioactive compounds produced by the plants for their protective mechanism. These phytochemicals have significant antioxidant, antimicrobial, antidiarrheal, antiallergic, antispasmodic and antiviral activities. They also enhance the immunity and protect against lung and prostate cancers. Qualitative phytochemical analysis of Naruvili chooranam reveals the presence of Saponins, Tannins, Terpenoids, Alkaloids, flavanoids and Glycosides.

SAPONINS

Saponins have multiple effects and are named as phytoanticipins or phytoprotectants. Biological effects of saponins such as Hypolipedemic, Hypoglycemic, Anti-inflammatory, Virucidal, antifungal, Anticancer, Immunomodulatory, Antibacterial, Analgesic, Immunomodulatory, Antiulcerogenic, Hepatoprotective.

TANNINS

Tannins possess antioxidant, Antibacterial, Antiparasitic, Anti inflammatory, Anti diarrheal, antiseptic activities.

TERPENOIDS

Terpenoids are the most abundant secondary metabolites in plants. Terpenoids possess antiulcer, antibacterial, antiviral, antimalarial, promotes transdermal absorption, prevent and treat cardiovascular disorders. Terpenoids possess complex structure with diverse effects and different mechanism of action.

ALKALOIDS

Alkaloids possess diverse physiological functions such as antibacterial, antimitotic, anti-inflammatory, analgesic, local anesthetic, hypnotic, psychotropic, antitumor activity.

FLAVANOIDS

Flavanoids are bioactive substances responsible for plant antioxidant and medicinal values. It is considered as the health promoting dietary constituent. Flavanoids compounds are shown to have an antioxidant activity, free radical scavenging capacity, cardioprotective, anti diabetic, anti-inflammatory, anti-allergic activity. Most recently flavanoids are prove to be the most effective as an anticancer agent, through apoptosis by induction of cell cycle arrest and inhibition of key enzymes involved in tumour presentation.

GLYCOSIDES

Glycosides are versatile family of natural chemicals with wide range of therapeutic activity. These include anti microbial, anti cancer, anti inflammatory, immunomodulatory, neuroprotective, urinary antiseptics, natural sweetener.

RESULT

Table 2: Quantitative analysis of Naruvili Chooranam.

Test	OD Value 1	OD Value 2	Mean OD Value	Result
Saponin	0.680	0.688	0.684	144 mcg/100mg
Tannin	0.248	0.225	0.237	27 mcg/100mg
Terpenoid	0.146	0.142	0.144	5 mcg/100mg
Alkaloid	0.323	0.333	0.326	98 mcg/100mg
Flavonoid	0.498	0.485	0.491	104 mcg/100mg
Glycoside	0.442	0.451	0.446	33 mcg/100mg

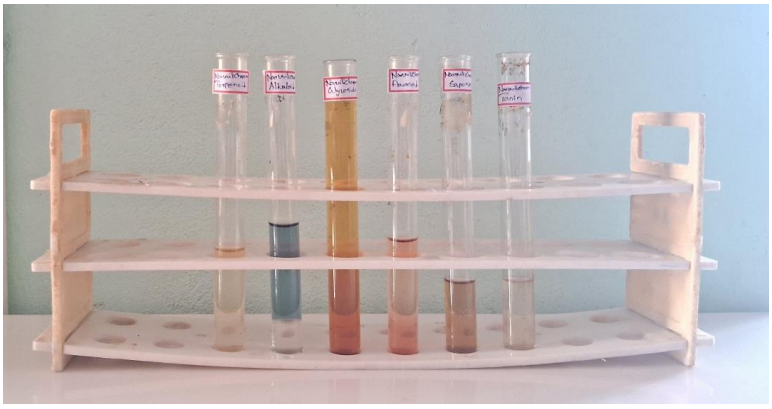

Figure 1: Quantitative analysis of Naruvili Chooranam.

DISCUSSION

Quantitative analysis accounts for the quantity or concentration of phytochemical in a plant sample. Quantitative analysis is more thorough and beneficial than qualitative analysis because the findings are valuable for various purposes such as drug discovery, herbal drug standardization, understanding the medicinal properties of plants and assessing the plant toxicity levels. Quantitative analysis showed each 100 mg of Naruvili chooranam contains 144mcg of Saponin, 27mcg of tannin, 5mcg of Terpenoids, 98mcg of Alkaloids, 104mcg of Flavanoids, 33mcg of Glycoside.

CONCLUSION

Naruvili plays an important role in treatment of Iraippu noi (Bronchial Asthma).

The preliminary phytochemical analysis showed the presence of tannins, Saponins, Alkaloids, Flavanoids, Glycosides, Terpenoids. It shows the efficacy of Naruvili chooranam in the management of Iraippu Noi. Further quantification of phytochemicals yields much more beneficial effect towards the potency of Naruvili chooranam which can be valuable for its standardization in the future.

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