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PRELIMINARY PHYTOCHEMICAL ANALYSIS OF ARUGAMPUL VER KUDENEER

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ABSTRACT
Recently there is a surge among people in choosing alternative system of Siddha, over modern medicine. Hence it is very much essential to standardize Siddha drug as specified by World Health Organization. **Aim**: In the present study we carried out phytochemical analysis of Siddha drug formulation Arugampul Ver Kudeneer chooranam. **Materials and Method**: It comprises of various for a numcer of disease. Arugampul ver kudeneer is a herbel drug which is indicated for many commam Genito urinary tract disorders in the text book, Gunpadam Mooligai Vaguppu. Arugampul Ver (Cynodon dactylon) plants and Seeds white pepper (Piper nigrum) Were collected from the locality of Tirunelveli. Tamil nadu and were dried under sunshade and ground into coarse powder and then mixed in a 4: 1 ratio respectively**. Method**: The extract was prepared with Arugampul Ver Kudeneer was to being phytochemical screening test for Tannin, Saponins, Terpenoids, Phenol, Steroids, quinones, glycosides, carbohydrates, proteins, alkaloids, Flavonids. **Result and discussion**: The Phytochemical analysis Siddha drug of Arugampul ver kudeneer Saponins, Terpenoids, Flavanoids, Glycosides, Carbohydrates, Proteins. The Qanitative analysis of Arugampul Ver Kudeneer contain Saponoin-30mcg/100mg, Terpenoids 41mcg/100mg, Alkaloids - 49mcg/100mg, Flavanoid-40mcg/100mg, Glycosides- 5mcg/100mg, Carbohydrates- 163mcg/100mg, Proteins-12mcg/mg. **Conclusion:** The phytochemical analysis study for Arugampul Ver Kudeneer shows presence of Saponins, Terpenoids, Alkaloids, Glycolides, Flavanoids, Carbohydrates, Proteins which responsible for its biological activity. This evidence based data provide voluable information is helpful to standardization of Arugampul Ver Kudeneer.

KEYWORDS: Siddha medicine, Cynodon dactylon, white pepper phytochemical Analysis.

INTRODUCTION
Siddha medicine including mostly plants which plays an important role in medicinal properties for both preventive and curative. Medicinal plants are richest bio resource of drug in traditional system of medicine. They also involving in treatment for many disease .these medicinal valves of plants are in some chemically active substance they produce a definite physiological action on the human body.

Kalladaippu is a common disease of Urinary tract which has the following symptoms: burning micturition, urinary tract obstruction, low back pain, and referred pain in genital organs and in tip of penis, abnormal deposits of urine. This can be correlated with Urolithiasis in the morden system of medicine. Gunapadam mooligai vaguppu has mentioned a treatment named ARUGAMPUL VER KUDENEER (A combination of Cynodon dactylon Linn.) plants and white pepper (a combuination of Piper nigrum Linn.) seeds which in indicated for Kalladaippu.

Plants can protect themselves from microbes, insects and environmental change by producing certain chemical or secondary metabolites which are non- nutritive, and they are called phytochemicals, the major groups of phytochemicals are Saponnins, Phenols, Terpenoids, Alkaloids, Flavanoids, Glycosides, Carbohydrates, proteins. The present study is to find the phytochemical constituents present in Arugampul Ver Kudeneer. Due to certain peoperties like Anti- microbial. Anti-inflammatory, Anti- oxidant, Anti- carcinogenic, the metabolized can establish direct or indirect protection against pathogens.

MATERIALS AND METHOD

Selection of drug

Arugampul Ver Kudeneer mentioned in Gunapadam mooligai Vaguppu (Pg. no.56) was selected for evaluating the phytochemical analysis.

Ingredience of Arugampul Ver Kudeneer

Arugampul Ver (Cynodon dactylon).
White pepper (Piper nigrum).

Preliminary Phytochemical Analysis

Qualitative Analysis of Arugampul VER Kudeneer

1. Test for Saponins

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

2. Test for Tannis

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

3. Test for Terpenoids

To few mg of extract in chloroform, add conc. H2SO4. 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 precence of phenol.

5. Test for Steroids (Liberman Burchard test)

To a few mg of the extract 2ml 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 cencetrated sulphuric acid. Appearance of red colour shows the presence of quinone.

7. Test for Glycosides

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

8. Test for Carbohydrate

To the sample solution added few drops of a- naphthol and 2-3 ml conc. H2SO4. The appearance of reddish violet or purple ring at the junction of two liquids indicates the presence of carbohydrates.

9. Test for Alkaloids (Dragendroff's Test)

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

10. Test for Flavanoid

To substance in alcohol add 10% NaOH or ammonia. Adrak yellou 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.

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 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 digitoxi.

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.

Estimation of total proteins

Protein content was estimated by the method of Lowry et al.[14] 1 ml of sample was mixed with 0.5 ml of 0.1 N sodium hydroxide and 5 ml of alkaline copper reagent. The mixture was incubated in room temperature for 30 minutes. Folin– Ciocalteau reagent, 0.5 ml was added and incubated again for 10 minutes at room temperature. The absorbance was read at 660 nm against a reagent blank. The estimation was done in triplicates and the results were expressed mcg/g sample.

Quantitative Estimation of carbohydrate

The total sugar content was estimated by Anthrone method (Roe, 1955). A known amount of the sample was taken, ground well with 80% ethanol and was centrifuged at 4000 rpm. From the supernatant, 0.5 ml was taken and 5 ml of anthrone reagent was added. The tubes were kept in a boiling water bath for 15 min. After that, they were kept in a dark room for another 15 minutes. The colour intensity developed was read in a spectrophotometer at 650 nm.

RESULT

Table 1: Qulalitative analysis of Arugampul Ver Kudeneer.

Tests	Result
Saponins	Present
Tannins	Absent
Phenols	Absent
Terpenoids	Present
Alkaloids	Present
Flavanoids	Present
Steroids	Absent
Glycosides	Present
Carbohydrates	Present
Quinones	Absent
Proteins	Present

Table 2: Quantitative analysis of Aragampulver kudineer.

Test	OD Value 1	OD Value 2	Mean OD Value	Result
Saponin	0.117	0.128	0.122	30 mcg/100mg
Terpenoid	0.563	0.568	0.565	41 mcg/100mg
Alkaloid	0.120	0.129	0.125	49 mcg/100mg
Flavonoid	0.169	0.175	0.172	40 mcg/100mg
Glycoside	0.071	0.078	0.068	5 mcg/100mg
Carbohydrate	0.873	0.867	0.870	163 mcg/100mg
Protein	0.033	0.026	0.029	12 mcg/100mg

DISCUSSION

The extract was prepared with Arugampul Ver Kudeneer drug was to being phytochemical screening test for Saponins, Phenols, Tannins, Terpinods, Alkaloids, Flavanoids, Streoids, Glycosides, Carbohydrates, Quinones, Proteins. The qualitative analysis of phytochemical screening of siddha drug of Arugampul ver kudeneer show the presence Saponins, Terpenoids, Alkaloids, Flavanoids, Glycosides, Carbohydrates, and Proteins. The quantitative analysis of Arugampul Ver Kudeneer containSaponins-30mcg/100mg, Terpenoids-41mcg/ 100mg, Alkaloids- 49mcg/100mg, Flavanoids-40mcg/100mg, Glycosides- 5mcg/100mg, Carbohydrates- 163mcg/mg, Protein 12mcg/100mg.

CONCLUSION

The qualitative analysis of phytochemical screening of siddha drug of Arugampul Ver Kudeneer shows the

presence Saponins, Terpenoids, Alkaloids, Flavanoids, Glycosides, Carbohydrates, and Proteins. The quanitative analysis of Arugampul Ver Kudeneer contains Saponins, terpenoids, alkaloids, flavonoids, glycosides, carbohydrates, and proteins in respectively in 30, 41, 40, 5, 163, 12mcg/100mg, are responsible for the biological activity. This evidence bassed data provide valuable information is helpful to standardization of Arugampul Ver Kudeneer.

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