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The Phyto - Chemical analysis of Siddha formulation *Aththikkai chooranam*

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Abstract

Herbal medicine, also with the synonyms such as Phyto-medicine, Botanical medicine is one of the sub-group of Complementary & Alternative Medicine (CAM) therapies. Preliminary standardization steps are essential for identification of genuine drug and setting analytical standards. Therapeutic potential of herbal drugs are attributed to the presence of phytochemicals. The study was performed to document the Phyto-chemical (both qualitative &quantitative) parameters of Siddha formulation *Aththikkai chooranam* (AC). Preliminary Phyto-chemical screening of AC established the presence of chemical constituents like Alkaloids, Glycosides, Terpenoids, Saponins, Tannins& Flavonoids. Quantitative analysis for Phyto-chemicals in aqueous and methanol extracts showed that Alkaloids (3.8 ± 0.1µg / ml), Glycosides (6.75 ± 0.2µg / ml), Terpenoids (1.9 ± 0.2µg / ml) and Saponins (7 ± 0.5µg / ml)were maximum quantifiable in methanol extraction whereas Tannins(14 ± 0.3µg / ml)and Flavanoids(18.5 ± 0.2µg / ml)in aqueous extraction .

Keywords: *Aththikkai chooranam*, Physico-chemical, Phyto-chemical analysis

I. Introduction

The medicinal plants have been using in all civilizations as a source of raw drug(s). Herbal medicine, also with the synonyms such as Phyto-medicine, Botanical medicine[3] is one of the sub-group of Complementary & Alternative Medicine (CAM) therapies. The traditional herbal medicine plays a major role in maintenance of public health in economically poor and developing countries. More-over in industrialized societies, medicinal plants are abundantly depended for the development of newer drugs and neutraceutical products. Therefore, preliminary standardization steps are essential for identification of genuine drug and setting analytical standards. The study was performed to document the Phyto-chemical (both qualitative & quantitative) parameters of single herbal formulation *Aththikkai chooranam* (AC).

II. Materials

2.1 Collection of raw drug:

The raw drug was freshly collected from Western Ghats (Trivandrum), Kerala. Then proper identification and authentication was done at Department of Medicinal Botany, Government Siddha Medical College & Hospital, Palayamkottai, Tirunelveli district, Tamil nadu.

2.2 Purification & preparation of AC:

Stalks and unnecessary parts were removed. It is then dried in shade and processed to obtain micronized powder. Finally the powder was sieved using pure white cloth which is mentioned as *Vasthirakayam* in *Siddha*. Finally stored in a clean and air tight container.

III. Methods

3.1Test procedure:

The Qualitative estimation of phyto-chemicals were conducted at Siddha Regional Research Institute, Poojappura, Trivandrum, Kerala. The Quantitative estimation of phyto-chemicals was conducted at Inbiotics, William hospital campus, MS road, Nagercoil, KK district, Tamil nadu.

3.2 Qualitative estimation of phytochemicals[5]

5g of AC was taken in a 250ml clean beaker and 50 ml of distilled water was added, boiled well and allowed to cool. It was filtered in a 100ml volumetric flask and made up to 100ml with distilled water. The AC was subjected to following screenings.

Terpenoids

Noller's test: Warm the substance with 2 or 3 tin bits and 2ml ofthionyl chloride.

Flavones

Shinado's test: To the substance in alcohol, a few magnesium turnings and a few drops of conc. HCl was added and boiled over a water bath for two minutes.

Steroids

Liebermann Burchard test: Dissolve the substance in 2 ml of chloroform, 1 ml of acetic anhydride and 1 ml of glacial acetic acid. Warm and cool under tap. 2 drops of conc. H_2SO_4 was added along the sides of the test tube.

Quinones

To the test substance, NaOH was added.

Anthraquinones

Borntrager's test: To the substance aqueous ammonia or caustic soda was added.

Glycosides

Mix the substance with a little anthrone on a watch glass, then one drop of conc. H_2SO_4 was added and make into a paste and warm gently over a water bath.

Alkaloids

To the test substance, 1 ml of dragendroff's reagent and 1% H_2SO_4 was added.

Tannins

Substance was treated with alcoholic and lead acetate solution.

Saponins

Substance was diluted separately with 20ml of distilled water and it was agitated on a graduated cylinder for 15 min.

Amino acids

Treat the substance in alcohol or water with ninhydrin in alcohol.

3.3 Quantitative estimation of phytochemicals

Alkaloids [2]

To 1ml of Methanolic extract, 5 ml pH 4.7 phosphate Buffer and 5 ml BCG solution were added. Shake the mixture with 4 ml of chloroform and extracts were collected in a 10-ml volumetric flask. Then it was 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 equivalents

Glycosides [7]

10ml of the extract and 10ml of Baljet's reagent were taken and allowed to stand for one hour. Then the solution was diluted with 20ml distilled water. Read the intensity of the color obtained against blank at 495nm using a spectrophotometer. The difference between test and control is taken for calculation. Standard graph was prepared using standard digitoxin.

Saponins [2]

Aqueous extract was dissolved in 80% methanol, 2ml of 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 ^{o}C for 10min.Absorbance was measured at 544nm against reagent blank. Diosgeninis was used as a standard material and compared the assay with Diosgenin equivalents.

Terpenoids [1]

Centrifuge the samples (4000g for 15 min. at room temperature) & collect the supernatant in a fresh 2 ml micro-tube. Add 1.5 ml Chloroform in each 2 mlmicrocentrifuge tube & then add 200µl sample supernatant in each. REMARK For the standard curve 200µl of previously prepared Linalool solution in methanol will be added to 1.5 ml Chloroform & serial dilution must be done[dilution level-100mg/200µl to 1mg/200µl(12.965 µM- 1.296 µM) Linalool Conc. In case of serial dilution total volume of 200µl will be make up by addition of 95% (Vol/Vol) Methanol].

Vortex the sample mixture thoroughly & take the time 3 min to rest. Add 100µl Conc. Sulfuric acid (H_2SO_4) to each 2 ml microcentrifuge tube. **Critical step**: If heat generation occurs then the entire system must be cooled by using ice-pad but not more than 15 min. Then the assay tube must be incubated at room temperature for 1.5h-2h in dark. **Pause point:** For standard solution (Linalool) incubate not more than 5 minutes and during incubation time the microcentrifuge tube must not be disturbed. At the end of incubation time a reddish brown precipitation will be formed in each assay microcentrifuge tube. Then carefully & gently decant all supernatant reaction mixture liquid with-out disturbing the precipitation. **Critical step:** The reddish brown precipitation is partially soluble in reaction mixture solution so must gently decant the supernatant fluid. Add 1.5 ml of 95% (Vol/Vol) Methanol & vortex thoroughly until all the precipitation dissolve in Methanol completely. Transfer the sample from assay tube to Colorimetric cuvette [95% (Vol/Vol) Methanol will be used as blank] to read the absorbance at 538 nm. Calculate a standard curve from the blank-corrected at wave length at 538nm of the Linalool standard. Calculate total terpenoids concentration of unknown plant sample as Linalool equivalents using the regression equation of Linalool standard curve.

Tannins [4]

1ml 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 mixture was allowed to stand for 20mins and the absorbance was measured at 500nm. The standard graph was plotted for working standard catechin solution (0 to 250µg/µl).

Flavanoids [2]

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 and 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).

IV. Results and Discussion

Qualitative estimation of phytochemicals revealed the presence of Terpenoids, Flavonoids, Glycosides, Alkaloids, Tannins and Saponins (Table 1).

Table 1 - Qualitative estimation of phytochemical constituents [6]

Qualitative phytochemical analysis	Observation	Results
Terpenoids	Magenta colour formed	Present
Flavanoids	Magenta or red colour formed	Present
Steroids	No blue green colour formed	Absent
Quinones	No red colour formed	Absent
Anthraquinones	No pink colour in the aqueous layer	Absent
Glycosides	Dark green formed	Present
Alkaloids	Orange- red precipitation	Present
Tannins	Bulky precipitation	Present
Saponins	Foam formed	Present
Amino acids	No blue to pink colour change	Absent

Table 2 - Quantitative estimation of phytochemical constituents in aqueous extract

Test	Values
Alkaloids µg / ml	1.2 ± 0.1
Glycosides µg / ml	3.6 ± 0.2
Terpenoids µg / ml	1.1 ± 0.2
Saponin µg / ml	2.35 ± 0.4
Tannins µg / ml	14 ± 0.3
Flavonoids µg / ml	18.5 ± 0.2

The quantitative estimation results listed in tables 2 & 3 showed that Alkaloids, Glycosides, Terpenoids and Saponins were maximum quantifiable in methanol extraction whereas Tannins and Flavanoids in aqueous extraction

Table 3 - Quantitative estimation of phytochemical constituents in methanol extract

Test	Values
Alkaloids µg / ml	3.8 ± 0.1
Glycosides µg / ml	6.75 ± 0.2
Terpenoids µg / ml	1.9 ± 0.2
Saponin µg / ml	7 ± 0.5
Tannins µg / ml	6.25 ± 0.1
Flavonoids µg / ml	7 ± 0.2

V. Conclusion

Analytical tests are essential tool for authentication, standardization and quality control assessment of raw drugs. Selection of such genuine drugs in the manufacturing of traditional medicines are need of the hour. The results revealed that AC has significant presence of Alkaloids (3.8 ± 0.1µg / ml), Glycosides (6.75 ± 0.2µg / ml), Terpenoids (1.9 ± 0.2µg / ml) and Saponins (7 ± 0.5µg / ml) in methanol extraction whereas Tannins (14 ± 0.3µg / ml)and Flavanoids (18.5 ± 0.2µg / ml)in aqueous extraction The above phytochemicals will be very effective to cure type 2 DM and its complications.

References

1. Bhalla, K.N et al. 'Microtubule-targeted anticancer agents and apoptosis'. Oncogene. 22, 9075-9086 (2003).
2. Devanaboyina N et al., "Preliminary Phytochemical Screening, Quantitative Estimation And Evaluation Of Antimicrobial Activity Of *Alstonia macrophylla* Stem Bark" IJSIT, 2013, 2(1), 31-39
3. Hira choudary et al, 'An update on natural compounds in the remedy of diabetes mellitus: A systematic review', Journal of traditional and complementary medicine 8 (2018), 361-368
4. Robert, EB, "Method for estimation of tannin in grain sorghum ", Agro J , *vol.* 63, 1971,p.511*;* 10.
5. Solich P, Sedliakova V, Karlicek R et al. Spectrophotometric determination of cardiac glycosides by flow-injection analysis. Anal ChimActa. 1992; 269(2): 199-203.
6. Sumayya et al, 'Qualitative phytochemical analysis and anti-microbial activity of siddha herbal preparation nellikudineer (Amla), ejpmr,2019,6(6), 529-533
7. Tiwari P, B.Kumar, M.Kaur, Int. Pharmaceut. Sci, 2011,1, 98-106

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