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<p>Phytochemical analysis of the Siddha herbal formulation Aavaraivithaadhi Chooranam</p>		
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<p>History of Article:</p> <p>Received 2 October 2015 Received in revised from 10 October 2015 Accepted 13 October 2015 Available online 25 October 2015</p>	<p style="text-align: center;">ABSTRACT</p> <p>Our Mother Nature has nurtured the mankind from time immemorial. Siddha medicine is a unique one as it is not only a curative but also preventive and to achieve the healthy body and mind. Siddha medicines revitalize and rejuvenate the body. The drug Aavaraivithaadhi Chooranam has Aavarai seed (<i>Cassia auriculata</i> seeds) as a key ingredient of the drug followed by nine herbal juices in which it is soaked. The Phytochemical screening of the extract gives general idea regarding the nature of chemical constituents present in the crude drug. The phytochemical analysis reveals the presence of alkaloids, tannins, flavonoids, anthral glycosides, cardiac glycosides, saponins, phenols, proteins and carbohydrates. From the above study it is concluded that the siddha herbal formulation AVC shows the presence of phytochemicals. A synergistic effect of all these flavonoids, alkaloids, glycosides, tannins, phenols, saponins increases the potency of the drug.</p> <p>Keywords: <i>Cassia auriculata</i>, Chooranam, Phytochemicals, Flavonoids.</p>	

INTRODUCTION

Our Mother Nature has nurtured the mankind from time immemorial. Siddhars are the Spiritual Scientists who unraveled the mysteries of Mother Nature. Human being is a part of nature therefore Siddhars have found remedy from nature which paved the way for dawn of the Siddha system.

Siddha medicine is a unique one as it is not only a curative but also preventive and to achieve the healthy body and mind. Siddha medicines revitalize and rejuvenate the body. Siddhars have contributed numerous pharmaceutical preparations through their literature which has been written in the palm leaves. Herbal formulations are given prime priorities than

herbo-mineral and mineral drugs in treating diseases.

According to Siddha Materia Medica the medicines are obtained from herbs, metals, minerals and animals products (Thiyagarajan.R, 2008). This paper explains about the study of *Cassia auriculata* L. (Cesalpinaceae, common name: Tanner's Cassia) a common plant in Asia. It has been widely used in traditional system of medicine for treating rheumatism, conjunctivitis and diabetes^[4]. The drug Aavaraivithaadhi Chooranam has Aavarai seed (*Cassia auriculata* seeds) as a key ingredient of the drug followed by nine herbal juices in which it is soaked.

Chooranam is a fine powder of drugs. The "Chooranam" may be applied to the powders of a single drug or a mixture of two or more drugs, which are powdered separately prior to

their being mixed to homogeneity. (Formulary Of Siddha Medicine, 1993) Then chooranam is said to retain its potency for 2 months and then gradually deteriorate. However if properly packed & stored they keep good for a year. (Formulary of Siddha Medicines, 1993).

The drug Aavaraivithaadhi Chooranam being an herbal formulation has a high amount of phytochemicals. Thus the aim of the study is the screening of phytochemicals present in the drug AVC.

MATERIAL AND METHODS

The siddha herbal preparation Aavaraivithaadhi Chooranam was selected from the Classical Siddha literature (Sarabenthirar Vaidhiya Muraigal-Neerizhivu Chiikitchai, 1992).

COLLECTION, IDENTIFICATION AND AUTHENTICATION OF THE DRUG

Each and every plant materials were freshly collected from in and around Trichy, Tamilnadu. They were identified and authenticated by the Botanist and *Gunapadam* experts at Government Siddha Medical College, Arumbakkam, and Chennai – 106. The specimen sample of all the herbs have been preserved in PG *Gunapadam* department individually for future reference.

PURIFICATION OF THE DRUGS

All the ingredients of this herbal formulation were purified according to the proper procedure methods described in Siddha classical literature (Sarakku Suthi Muraigal, 2008) (Kannusamy pillai C, 1991).

PREPARATION OF THE DRUG

The seeds of *Cassia auriculata* were soaked and dried each day in the juices of 1 to 9 respectively. Then the seeds were dried in the shade until complete evaporation of the moisture content. It was finely powdered and kept in an air tight container. It was labeled as Aavaraivithaadhi Chooranam (AVC). Then the Chooranam was purified by steam boiling process according to the Siddha classical text (Ramachandran S.P, 1994).

PHYTOCHEMICAL ANALYSIS

The Phytochemical screening of the extract gives general idea regarding the nature of chemical constituents present in the crude drug. The phytochemical tests were done as the method

illustrated in (Prashant Tiwari et al., 2011 and Harborne, 1973).

Test for Alkaloids

A small portion of solvent free extracts were stirred separately with few drops of dilute hydrochloric acid and filtered & tested carefully with various alkaloidal reagents.

Mayer's reagent - Cream precipitate

Dragendorff's reagent - Orange brown precipitate

Hager's reagent - Yellow precipitate

Wagner's reagent - Reddish brown precipitate

Test for Carbohydrates and Reducing Sugars

The minimum amount of extracts were dissolved in 5ml of distilled water & filtered. The filtrate was subjected to test for carbohydrates & glycosides.

a) Molisch's test

The filtrate 1 ml was treated with 2-3 drops of 1% alcoholic alpha naphthol & 2ml concentrated sulphuric acid was added along the sides of test tube. Violet ring was observed at the junction of 2 layers which showed the presence of carbohydrate.

b) Benedict's test

The filtrate 1 ml was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c) Fehling's test

The filtrate 1 ml was treated with equal volume of Fehling's solution A and B and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Test for Glycosides

The extract was hydrolyzed with dil. HCl and subjected to test for glycosides.

a) Modified Bortrager's test

To the hydrolysate extract, 1 ml of Ferric chloride solution was added and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in the ammoniacal layer indicates the presence of Anthranol glycosides.

b) Legal's test

The hydrolysate extract was treated with Sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of Cardiac glycosides.

Test for Saponins

The extract 0.5 ml was shaken with 5 ml distilled water. The presence of saponins was indicated by formation of copious lather.

Test for Tannins

Gelatin test

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Test for Phenolic compounds

To 0.5 ml of extract, 1 ml of alcoholic ferric chloride solution was added. Formation of bluish green or bluish black indicates the presence of Phenolic compounds.

Test for Phytosterol

Ferric chloride – acetic acid test

1 ml of extract is treated with 1 ml of chloroform and then, 2 ml of ferric chloride acetic acid reagent is added followed by 1 ml of conc. sulphuric acid. Appearance of reddish pink colour shows the presence of phytosterol.

Test for Diterpenes

Copper acetate test

1 ml of extract was dissolved in water and treated with 3-4 drops of Copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Test for Triterpenes

Salkowski's test

1 ml of extract is treated with 1 ml of chloroform followed by 1 ml of conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour shows the presence of triterpenes.

Test for Flavonoids

a) Alkaline reagent test

To 1 ml of extract, 1 ml of 10% sodium hydroxide solution was added. Formation of dark yellow colour indicates the presence of flavonoids.

b) Lead acetate test

To 1 ml of extract, 3-4 drops of 10% lead acetate solution was added. Formation of yellow precipitate indicates the presence of flavonoids.

c) Ferric chloride test

To 1 ml of extract, 3-4 drops of ferric chloride solution was added. Formation of dark green colour indicates the presence of flavonoids.

d) Shinoda test

To 1 ml of extract, few mg of magnesium turnings was added followed by few drops of conc. hydrochloric acid and boiled for five minutes in a boiling water bath. Formation of red colour indicates the presence of flavonoids.

Test for Proteins and Free Amino Acids

a) Xanthoproteic test

To 1 ml of extract, 3-4 drops of conc. nitric acid was added. Formation of yellow precipitate indicates the presence of proteins.

b) Million's test

To 0.5 ml of extract, 2.5 ml of Million's reagent was added. Formation of white precipitate and the precipitate warmed indicates the presence of proteins.

c) Biuret test

To 0.5 ml of extract, 2.5 ml of diluted Biuret reagent was added. Appearance of purple colour or brick red precipitate showed the presence of proteins and free amino acids.

Test for Quinones

Sodium hydroxide test

To 0.5 ml of extract, 1 ml of 10% sodium hydroxide was added. Appearance of blue or green or red colour shows the presence of quinones.

Table-1. Phytochemicals screening test

Phytochemicals	Test	Result
1. Alkaloids	a. Mayer's test	++
	b. Wagner's test	-
	c. Dragendorff's test	-
	d. Hager's test	-
2. Carbohydrates	a. Molisch's test	+
3. Reducing sugars	a. Benedicts test	-
	b. Fehling's test	-
4. Anthranol Glycosides	Modified Borntrager's test	+
5. Cardiac glycosides	Legal's test	+++
6. Saponins	a. Froth test	+
	b. Foam test	-
7. Tannins	Gelatin test	++
8. Phenols	Alcoholic Ferric chloride test	+
9. Phytosterols	Ferric chloride acetic acid test	-
10. Diterpenes	Copper acetate test	-
11. Triterpenes	Salkowski's test	-
12. Flavanoids	Alkaline reagent test	+++
	b. Lead acetate test	-
	c. Ferric chloride test	-
	d. Shinoda test	+
13. Proteins	a. Xanthoproteic test	-
	b. Biuret's test	+
	c. Million's test	-
14. Quinones	a. Sodium hydroxide test	-

RESULT AND DISCUSSION

Phytochemicals are natural bioactive compound found in plants and fibers which act as a defense system against diseases and more accurately, to protect against diseases. (James W, Anderson MD (1983). The phytochemical analysis reveals the presence of alkaloids, tannins, flavonoids, anthral glycosides, cardiac glycosides, saponins, phenols, proteins and carbohydrates.

Flavonoids

It is the most important group of poly phenolic compounds in plants. Flavonoids have

potent Anti-Oxidant activity and it is its important function. Oxidative stress plays a vital role in the pathogenesis of diabetes mellitus. Flavonoids are potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity (Salah et al,

1995; Del-Rio et al., 1997 and Okwu et al, 2004). Flavonoids can exert their Anti-Oxidant activity by scavenging the free radicals, by chelating metal ions or by inhibiting enzymatic systems responsible for free radical generation. It also possesses anti-microbial activity which is confirmed by the various anti-microbial assays.

Cardiac glycosides

They are known to reduce the complications of Diabetes Mellitus. They also protect heart from complications.

Tannins

Reported to have anti-diabetic activity. They restore the Anti-Oxidant status of the organs to almost normal levels. Increases the cellular Anti-Oxidant enzymes. Helps in healing of wounds and inflammation of mucous membrane.

Phenols

Effective Anti-Hyperglycemic agent. They possess rich Anti-Oxidant property and protect body from oxidative stress. Phenol groups are the essential part of many anti-oxidant compounds

Alkaloids

Alkaloids possess antispasmodic, analgesic, bactericidal effects. Alkaloids are the active principles producing many essential effects in protecting the body.

CONCLUSION

From the above study it is concluded that the siddha herbal formulation AVC shows the presence of phytochemicals. A synergistic effect of all these flavonoids, alkaloids, glycosides, tannins, phenols, saponins increases the potency of the drug. Thus the Siddha formulation AVC which is a combination of many herbal compounds contains many phytochemicals. So it increases the drug efficacy and potency in treating the diseases. Further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism of action of AVC and the drug bear the potential for further research.

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