ISSN No:-2456-2165

Investigation of Phytochemicals, Total Phenols and Total Flavonoids Content of Two Anti-Arthritic Plants

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Abstract:- Phytochemical investigation, total phenols and total flavonoids content of Strobilanthesciliatus root and Calophylluminophyllum leaves was carried out in the present inquiry. Various extracts of Strobilanthesciliatus root was prepared by sequential extraction separately with solvents ranging from polar to non-polar. Methanolic extract of Calophylluminophyllum leaves was prepared by maceration. The extracts were subjected to phytochemical screening, total phenols and total flavonoids estimation by using standard procedures.Total phenols and flavonoids content of methanolic extract of both the plants were comparatively higher and this may be due to the phytochemicals present in these extracts.

Keywords:- *Strobilanthesciliatus, Calophylluminophyllum, phytochemical screening, total phenols, total flavonoids.*

I. INTRODUCTION

Natural sources such as plants have received considerable attention for discovery and development of leads as new drug molecules, because of its diversity. Strobilanthesciliatus Wall. *ex* Nees of Acanthaceae family is a extremely promising medicinal plant in Ayurveda, used in the treatment of inflammatory disorders (Thomas*et al*., 2000). The plant is used for a variety of ailments like rheumatalgia, lumbago, sciatica, limping, chest congension, strangury, fever, leucoderma, skin diseases, inflammations, cough, bronchitis, odontalgia and general debility (Warrier*et al*., 1994).The roots are bitter, sweet, causes production of heat, emollient, diurectic, febrifuge, diaphoretic,depurative, anti inflammatory, expectorant and tonic. CallophylluminophyllumL. of Clusiaceae family has been routinely used for the treatment of rheumatism, skin diseases, dysentery and bleeding piles (Nadkarni, 1954). The entire plant is medicinal and carries compounds such as xanthones, triterpenes, coumarins and glucosides. The anti-inflammatory effect of *C. inophyllum*was reported earlier (Saxena*et al.*,1979).

II. MATERIALS AND METHOD

A. Plant collection:

The roots of Strobilanthesciliates were collected from Ranny, Pathanamthitta, Kerala. The leaves of Calophylluminophyllum were collected from Changanacherry, Kottayam, Kerala. Both the plant specimens were authenticated by Dr. Vinod kumar T.G., St. Thomas College, Ranny.

B. Extraction of plant material:

The roots of Strobilanthesciliates were thoroughly washed, shade dried, powdered (1kg) and was subjected to sequential extraction separately with solvents ranging from polar to non-polar in a Soxhlet extractor. The extracts were concentrated to dryness. The obtained extracts were kept in desiccators to remove moisture and stored properly until used. The extracts were administered to qualitative phytochemical investigation for recognition of various phytochemicals. Determination of tot.phenols content was done using FC reagent and tot.flavonoids content was approximated using $AlCl_3$method.

The leaves of Calophylluminophyllum were thoroughly washed, shade dried and roughly powdered (750 gm). The powder was macerated with methanol in a round bottom flask for 7 days. To ensure the efficiency of the extraction the contents of flask were stirred intermittently. The essence was filtered and the filtrate was evaporated. The procured extract was kept in desiccators to abolish moisture and stored properly until used. The extract was subjected to qualitative phytochemical investigation for identification of various phytochemicals. Determination of tot.phenol content was done using FC reagent and tot. flavonoid content was approximated using $AlCl_3$ method.

III. PHYTOCHEMICAL INVESTIGATION:

Phytochemical investigation was conducted as per standard procedure. (Trease and Evans, 1983; Harborne, 1973).

A. Test for alkaloids:

Warmed a small amount of various extract with 8 ml of 1% hydrochloric acid separately, and filtered. The resultant filtrate were treated separately with Maeyer's [Potassium mercuric iodide solution] and Dragendorff's reagents [Potassium bismuth iodide solution]. The presence of cream coloured precipitate for Maeyer'stest or reddish brown precipitate for Dragendroff's test indicated the presence of alkaloids.

B. Test for Glycosides:

In Killer-Killani test, Gl. acetic acid containing traces of $FeCl_3$ and concentrated $H_2SO_4$are added to each of the extract and observed for formation of red- brown color at the junction of two layers and blue green color at the upper layer indicated the presence of glycoside.

In Borntrager's test, benzene and few drops of dilute NH_3solution are added to the extracts and noticed for formation of red-pink color.

- *Legal test:* To the concentrated extract added few drops of 10% sodium hydroxide solution to make it alkaline

ISSN No:-2456-2165

and then freshly prepared sodium nitroprusside solution were added and observed for the formation of blue color.

- *Baljet test:* The concentrated extract was added with sodium picrate reagent and examined for orange and yellow color.

C. Test for Phenolic compounds:

In $FeCl_3$test, the extract was taken in water and heated mildly; to this $FeCl_3$ solution was added and examined for the generation of green and blue color.

In $(CH_3COO)_2$Pbtest:$(CH_3COO)_2Pb$ solution was combined to the extract and examined for the formation of precipitate.

- *Gelatin test:* to the aqueous extract added a few ml of Gelatin Solution and examined for precipitate or turbidity.

D. Detection of Flavonoids:

In NH_3 test, strips of filter paper are dipped in alc. extract solution, ammoniated, examined for color change.

- *Shinoda test (Magnesium Hydrochloride reduction test):*

Few fragments of Magnesium ribbon and conc. hydrochloric acid were added drop wise to each of the extract, a pink scarlet, crimson red or occasionally green to blue color appears after few minutes if flavonoids are present.

E. Test for Reducing sugars:

- *Molisch test*:

Small quantities of extracts were dissolved individually in distilled water followed by filtration. 2-3 drops of α-naphthol solutions were added followed by conc.H_2SO_4 along the sides of tilted test tube. A violet colored ring at the junction of two layers showed a positive test.

F. Test for terpenoids:

- *Noller's test*: Into a dry test tube few mg of extract was taken and is treated with a bit of tin foil and few drops of thionyl chloride and was heated gently. The formation of pink colour indicates terpenoids.

G. Test for Sterols:

In Libermann-Buchard test, the different extracts were treated with few drops of $(CH_3CO)_2O$ and then boiled and cooled. Through the sides of the test tube, conc. H_2SO_4 was added. At the junction of two layers appearance of brown ring and green color at the upper layer indicates steroids.

In Salkowski test, extracts are reacted with $CHCl_3$ and few drops of conc. H_2SO_4, mixed well, kept for some time. Steroids showed a red color at the bottom layer.

H. Foam test for Saponins:

The plant extracts were added with few ml of distilled H_2O and mixed heavily for a stable persistent froth. The presence of saponins was indicated by the appearance of froth.

I. Determination of total phenolics and flavonoids

- *Determination of total phenols*:

(Li Fu *et al.*, 2010) FC reagent was used for approximation of total phenols. In a series of test tubes, each extract in methanol was taken and mixed with FC reagent (1:10 diluted) and after 4 minutes, satd.Na_2CO_3 solution was added. After shaking, it was kept at room temperature for 2 h, and the absorbance was measured at 760 nm versus prepared water blank. Gallic acid monohydrate was used as a standard compound for the quantification of total phenols. The total phenols content was calculated using the standard curve, and expressed as gallic acid equivalent in mg/g of extracts.

- *Determination of total flavonoids*:

(Zhishen*et al.*, 1999) The total flavonoids content of samples was determined by following the Aluminium chloride method. Plant concentrate was mixed with distilled H_2O and $NaNO_2$ solution. After 6 min, $AlCl_3$ solution was added and enabled to stand for 6 min, NaOH solution was added to the mixture. Immediately distilled H_2O was added to bring to the final volume and then the mixture was extensively mixed and enabled to stand for another 15 min. Optical density of the mixture was recorded at 510 nm. Rutin was used as a standard compound for the evaluation of total flavonoid. The total flavonoids were calculated using the standard curve, and expressed as rutin equivalent in mg/g of extracts.

IV. RESULTS AND DISCUSSION

The total phenol and flavonoid content of methanol extract of Strobilanthesciliatus were found to be higher when compared to other extracts as indicated in Table 1. From phytochemical screening of Strobilanthesciliatus the methanolic extract is found to have different phytoconstituents like Phenolics, Flavonoids, Glycosides, Terpenoids, Sterols and saponins. Methanolic extract of Calophylluminophyllum also showed marked total phenol and total flavonoid content. Phytochemical screening of methanolic extract of Calophylluminophyllum revealed the occurrence of phytochemicals like Alkaloids, Flavanoids, Reducing sugar, Terpenoids and saponins. Therefore, the methanolic extracts of both the plants can be selected for further investigations of these plants for various pharmacological activities.

ISSN No:-2456-2165

Extract	Extraction			
	Characteristics	Phytochemcalexamination	Total phenol mg/g [a,b]	Total flavonoid mg/g [a,c]
Strobilanthesciliatus				
Petroleum ether extract	Dark green sticky residue	Terpenoids	-	-
Chloroform extract	Blackish green sticky residue	Alkaloids, Flavonoids, reducing sugar	64.52 ± 1.51	15. 53 ± 0.90
Ethyl acetate extract	Brownish semisolid residue	Phenolics, Flavonoids, Terpenoids, saponins	76.23 ± 2.15	29.80 ± 1.72
Methanol extract	Brownish residue	Phenolics, Flavonoids, Glycosides, Terpenoids, Sterols, saponins	99.34 ± 5.83	38.43 ± 1.92
Aqueous extract	Brownish residue	Phenolics, Flavonoids, Sterols, saponins	81.64 ± 0.84	26.49 ± 2.08
Calophylluminophyllum				
Methanol extract	Black semisoild residue	Alkaloids, Flavanoids, Reducing sugar, Terpenoids, saponins	89.28 ± 1.64	39.58 ± 3.23

Table 1. Extraction, Phytochemical examination, Total Phenol and Total Flavonoid Estimation of Strobilanthesciliatus and Calophylluminophyllum

a) Mean of 3verifications, mean ± SEM
b) Gallic acid ≡ mg/g extract
c) Rutin≡mg/g of the extract

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