Invitro Anti Inflammatory Activity of Hydro Ethanolic Extract of Cathium Coromandelicum By Using Protein Denaturation And Protein Inhibitatory Method

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Abstract- *Natural plants contain medicinal properties and their pharmacological activity. Plant have hung amount of phytoconstituents such as alkaloids, flavonoids, terpenoids, phenol and tannins. Hydroethanolic extract of Canthium Coromandelicum leaf extract contains flavonoids, terpenoids, finally conclude that plant has anti-inflammatory activity. The plant of Canthium Coromandelicum has proved that anti-inflammatory activity for hydroethanolic extract by protein denaturation and proteinase activity method. Invitro anti-inflammatory activity measured by using graphical method. Protein denaturation and Protein inhibitory using standard for Diclofenac sodium and compare with plant sample. This was further confirmed by comparing their IC50 values. Performing for the protein denaturation by using hydroethanolic extract possessed IC50 value 370µg/ml, where as that of Diclofenac sodium was found 300 µg/ml. Another test performing for proteinase activity by hydroethanolic extract possessed IC50 value 400 µg/ml, where as that of Diclofenac sodium was found 310 µg/ml. Therefore our aim research finally conclude that, plant of Canthium Coromandelicum have a querying the inflammation.*

Keywords- Canthium Coromandelicum, flavonoids, Diclofenac sodium, anti-inflammatory, Protein denaturation, Protein inhibitory.

I. INTRODUCTION

Medicinal plants have wild types of medicinal uses and their therapeutic effects. Plants have a many types of phytoconstituents that can provide pharmacological activity. Now days have many plants using for anti-inflammatory activity. During 1950 -1970 produced many drug such as deserpidine, reseinnamine, vinblastine (Sirigiri Chanda Kala 2015). Plant based drug are less side effect, but chemical drug has harmful side effects (Chidambaram *et al*., 2018). *Canthium Coromandelicum* belongs to family of Rubiaceae. All plant parts such as roots, leaves, barks, fruits are querying many disease (Chidambaram 2018). The plant of *Canthium Coromandelicum* widely available in some country such as India, South China and Srilanka (Karthick *et al*., 2014). *Canthium Coromandelicum* leaf are used for intestinal worms, ring worm, diarrhea, fever, leucorrhoea, anthelmintic, febrifuge (Chidambaram 2018). Hydroethanolic extract of *Canthium Coromandelicum* has a rich in bioactive compounds such as alkaloids, flavonoids, terpenoids, phenol and tannins. The present study was revealed that Invitro anti-inflammatory activity against protein denaturation and protein inhibitory method

II. MATERAL AND MATHODS

Plant collection and authentication

Leaves *Canthium Coromandelicum* was collected from nambiyur and authenticated (No: BSI / SRC/5/23/2017/Tech/912) at Botanical Survey of India (BSI), Coimbatore, Tamilnadu Agricultural University, Coimbatore.

I.Qualitative analysis of phytochemical in the hydroethanolic extract of the *Canthium Coromandelicum* (Chidambaram *et al*., 2018)

1) Test for Alkaloids

a)Mayer's test (Potassium mercuric iodine)

To a 0.5 ml of extract, two drops of Mayer's reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

b) Wagner's test (Iodine- potassium iodine solution)

A few drops of Wagner's reagent are added to 0.5 ml of extract along the sides of test tube. A reddish (or) Brown precipitate indicates the presence of alkaloids.

2) Test for Flavonoids

a)Alkaline reagent test

To 0.5ml of extract added few drops of sodium hydroxide solution. Intense yellow color which disappeared after adding dilute HCl indicates the presence of flavonoids.

3) Test for Saponins

a)Foam test

When small amount of extract was shaken with little quantity of water, persistence of foam appearance for about 10 minutes; symbolizes the presence of Saponins.

4) Test for Steroids

a) Salkowski test

Few drops of concentrated sulphuric acid were added with the extract, shaken and allowed to stand. Appearance of red colour in lower layer indicates the presence of steroids.

b)Libermann-burchara's test

To 1.0 ml of sample extract, 1.0 ml of concentrated H2SO4 was added followed by the addition of 2.0 ml of acetic anhydride solution. A greenish color developed and it turned blue to indicate the presence of steroids.

5) Test for Carbohydrates

a)Molish's test

To 2 ml of extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid are added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

b) Benedict's test

To 0.5 ml of extract, 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

c) Barfoed's test

To 1.0ml of extract, few drops of Basford's reagent was added and boiled in water bath. Brick red precipitate formation shows the presence of carbohydrates.

d) Fehling's test

0.5ml of extract were hydrolysed with dilute hydrochloric acid, neutralized with alkali and heated with equal amount of Fehling's A and B solutions. Formation of green to yellow to red precipitate indicated the presence of reducing sugars

6) Test for Tannins

a) Ferric chloride test

To 0.5ml extract a few drops of 1% neutral ferric chloride solution were added, formation of blackish blue color indicates the presence of tannins.

7) Test for Protein

a).Ninhydrin test

Heated the 3 ml of extract and 3 drops of Ninhydrin solution in boiling water bath for 10 minutes. Appearance of purple colour shows the presence of amino acids.

b) Biuret test

To 2 ml of extract added 4% NaOH and few drops of 1% Copper sulphate solution. Formation of violet colour confirms the presence of protein.

c) Million's reagent test

Mixed the extract with million's reagent. Formation of brick red precipitate indicates the presence of protein.

d) Xanthoprotein test

To 1 ml of concentrated nitric acid was added boiled for 1 minute and liquid ammonia was added. Precipitate formation indicates the presence.

8) Test for Glycosides

a) Born Trager's test

To 2 ml of extract hydrolysed, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates presence of glycosides.

9) Test for Phenol

a) Ferric Chloride test

The 2ml of extract is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenol.

b) Gelatin test

The 2ml of extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

c) Lead acetate test

The 2ml of extract to this 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

II. *IN VITRO* ANTI-INFLAMMATORY ACTIVITY OF HYDROETHANOLIC EXTRACTS OF THE LEAVES *CANTHIUM COROMANDELICUM*

1) Protein denaturation

A solution of 0.2% w/v of BSA was prepared in a Tris Buffer Saline and pH was adjusted to 6.8 using glacial acetic acid stock solutions of 10,000µg/ml of all the extracts were prepared by using ethanol as a solvent. From these stock solutions 4 different concentrations of 1,100, 200 and 500µg/ml were prepared by using ethanol as a solvent. 50µl (0.05ml) of each extract was transferred to Eppendorf tubes using 1ml micropipette. 5ml of 0.2% w/v BSA was added to the entire above Eppendorf tubes. The control consists of 5ml of 0.2% w/v BSA solution with 50µl ethanol. The standard consists of 100µg/ml of Ibuprofen in ethanol with 5ml 0.2% w/v BSA solution. The test tubes were heated at 72ºC for 5 minutes and then cooled for 10 minutes. The absorbance of these solutions was determined by using a UV-VIS Double beam spectrophotometer (ELICO SL 244) at a wavelength of 660nm. Each experiment was carried out in triplicate and the mean absorbance was recorded. The percentage of inhibition of precipitation was determined on a percentage basis relative to control using the formula (Kandikattu Karthick *et al.*, 2018).Percentage inhibition = 100 -(O.D. of test –O.D. of product control) x 100
O.D. of Control

2) Protein inhibition

The test was performed according to the modified method. The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100 - 500 µg/ml). The mixture was incubated at 37oC for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% per chloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control

III. RESULT AND DISSCUSSION

1) Percentage yield of different of *Canthium Coromandelicum*

The percentage yield of hydroethanolic extracts of *Canthium Coromandelicum* was determined according to the standard method. The percentage of dry extracts was calculated in terms of air dried weight. The hydroethanolic extract was found to produce percentage of yield (6.6%). Generally, a hydroethanolic extracts serves as best extract producing the % of dry weight.

2) Phytoconstituents in *Canthium Coromandelicum* leaf extract

The phytochemical screening of the plant extracts was shown in (table 1) and it revealed the presence of carbohydrates, proteins, alkaloids, flavonoids, phenols, steroids, Saponins, tannins and glycosides. The medicinal plant have lots of secondary metabolite that provide the physiological action to the body (Edogrul *et al.*, 2002).

TABLE: 1 phytoconstituents analysis of hydroethanolic extracts of the leaves *Canthium Coromandelicum*

TEST	AQUEOUS	PETROLEUM ETHER	CHLOROFORM	ETHYL ACETATE	HYDRO ETHANOL
Alkaloids	-	+	-	+	+
Flavonoids	+	-	+	-	+
Proteins	-	+	+	-	+
Phenols	+	-	-	-	+
Tannins	-	-	-	+	+
Saponins	+	-	-	-	+
Steroids	-	-	-	-	+
Terpenoids	+	-	+	-	+

From the results, compared with different solvent extracts such as aqueous, petroleum ether, chloroform, ethyl acetate and hydroethanolic extract.But finally all phytochemicals present in hydroethanolic extract, further research carry the hydroethanolic extract of *Canthium Coromandelicum*. All medicinal plants have a different

properties such as antioxidative, anti-inflammatory, antimicrobial (Brace *et al.*, 2002).

3) *In vitro* anti-inflammatory activity of hydroethanolic extract of *Canthium Coromandelicum*

1) Protein denaturation

Protein denaturation is a pathological process by which the proteins lose their configuration and become functionless (Opie, 1962). In the present investigation, the *in vitro* anti-inflammatory effect of *Canthium Coromandelicum* was evaluated against denaturation of egg albumin. The present findings exhibited concentration dependent inhibition of protein (albumin) denaturation by hydroethanolic extract throughout the concentration range of 100 to 500µg/mL Diclofenac sodium (at the concentration range of 100 to 500µg/mL) was used as reference drug which also exhibited concentration dependent inhibition of protein denaturation.

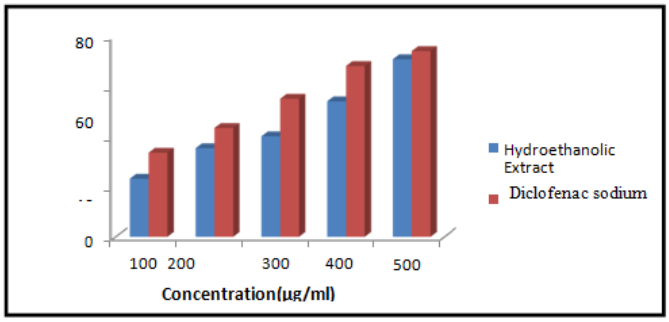

Figure:1Effectofhydroethanolicextractof*CanthiumCoromandelicum*onproteindenaturation activity

However, the effect of Diclofenac sodium was found to be moderate level, when compared with hydroethanolic extract of *Canthium Coromandelicum*. Figure 1 explain the standard (Diclofenac sodium) and sample (*Canthium Coromandelicum*) was explain bar graph.This was further confirmed by comparing their IC50 values. Hydroethanolic extract possessed IC50 value 370µg/mL where as that of Diclofenac sodium was found to be 300µg/mL The results of the present study was on par with the when compared with an earlier report of (Kamlesh pant et al., 2012) *in vitro* anti-inflammatory of *Anthracephalus cadamba* leaves extract.

3) Proteinase inhibitory action

The hydroethanolic extract exhibited significant anti-proteinase activity from leaf extract. The extract effectively inhibited the proteinase activity. The standard Diclofenac sodium drug showed the maximum proteinase inhibitory action. Proteinase has been implicated in arthritic reactions.

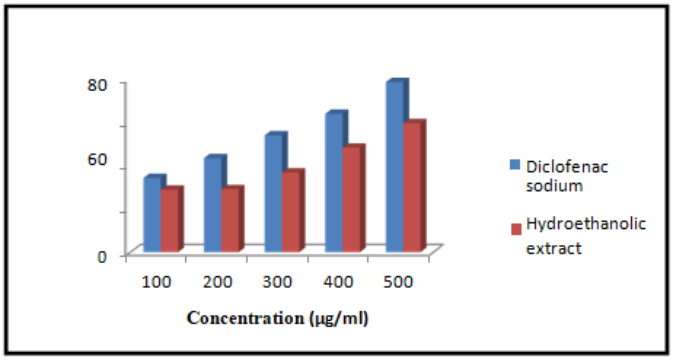

Figure:2 Effectofhydroethanolicextractof*CanthiumCoromandelicum*onProteinaseinhibitory activity

Figure 2 explain the standard (Diclofenac sodium) and sample (*Canthium Coromandelicum*) was explain bar graph. This was further confirmed by comparing their IC50 values. Hydroethanolic extract possessed IC50 value 400µg/mL whereas that of Diclofenac sodium was found to be 310µg/ml. It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by Proteinase inhibitors (Das *et al.*, 1995).However, the effect of Diclofenac sodium was found to be moderate level when compared with hydroethanolic extract.

IV.CONCULSION

Canthium Coromandelicum is valuablemedicinal plants and itsused for ayurvedic medicine. The hydroethanolic extract of *Canthium Coromandelicum* having variants types of bioactive compounds such asalkaloids, flavonoids, terpenoids, phenol and tannins.*The* aim of our study revealed thathydroethanolic extract of *CanthiumCoromandelicum*have anti-inflammatory activity by protein denaturation and Protein inhibitory method.

REFERENCES

[1] Sirigiri Chanda Kala (2015) A short Review of Rubiaceae species. International Journal of Pharma and Bioscience. 5(2):179 – 181.

[2] Chidambaramoorthy M, Brindha Durairaj, Santhi R (2018) Invitro Anticancer Potential of Hydroethanolic Extract of Canthium Coromandelicum against Neuroblastoma SHSY5Y cell line. International Journal for Science and Advance Research in Technology. 4(4): 2395 – 1052.

[3] Chidambaramoorthy M (2018) Review on Phytochemical and Pharmacological activity of Canthium Coromandelicum (Burm.f) Alston (Rubiaceae).

International Journal for Research and Development in Technology. 9(3): 2349 – 3585.

[4] Karthick VP, Srinivasan, Gowri, Punnagai (2014) Invitro free radical scavenging activity of Canthium Coromandelicum extract. World journal of Pharmacological research. 3(5): 1371 – 1376.

[5] Kandikattu Kathik, Bharath Rathma Kumar P, Venu Priya R, Sunsil Kumar K, Ranjith Singh . B. Rathore (2013) Evaluation of Anti – inflammatory of Canthium Parviflorum by *Invitro* method. Indian Journal of Research in Pharmacy and Biotechnology, 1(5): 2320 – 3471.