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IN VITRO ANTIOXIDANT AND ANTIARTHRITIS ACTIVITY OF EXTRACTS AND FRACTIONS OF CANTHIUM PARVIFLORUM

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ABSTRACT

Canthium parviflorum Lamk (Rubiaceae) is a shrubby and woody plant found throughout the Western Ghats. In siddha system of medicine the plant was used in respiratory disorder, diuretic, diabetic, rheumatism and obesity. Canthium parviflorum have reported to possess a number of pharmacological activities such as antioxidant, wound healing activity and antitumor acitivity. D-mannitol, phenolic acid, phenolic compounds, carbohydrates, proteins were found from Canthium parviflorum. The present invitro anti-arthritic and antioxidant study of the leaf of Canthium parviflorum was undertaken to substantiate its folkloric uses in the treatment of arthritis.

KEYWORDS: Canthium parviflorum, Rubiaceae, invitro anti-arthritis and anti oxidant activity.

1. INTRODUCTION

The primary advantage of in vitro work is that it permits an enormous level of simplification of the system under study, so that the investigator can focus on a small number of components. [1,2] For example, the identity of proteins of the immune system (e.g. antibodies), and the mechanism by which they recognize and bind to foreign antigens would remain very obscure if not for the extensive use of in vitro work to isolate the proteins, identify the cells

and genes that produce them, study the physical properties of their interaction with antigens, and identify how those interactions lead to cellular signals that activate other components of the immune system. [3] Cellular responses are species-specific, lending cross-species analysis problematic. Newer methods of same-species-targeted, multi-organ studies are available to bypass live, cross-species testing. [4]

In recent years, the emerging concepts in biology systems and the translation of these concepts into clinical trials drive the developments of in vitro diagnostics applications. Upon the comprehensive and emerging clinical needs, the probes for in vitro diagnostics are needed to be efficiently produced, highly sensitive, quantitative, rapid, handy, and even multiplexed to detect and monitor the biomolecules (e.g., DNA, RNA, and proteins) or bioentities (e.g., cancer cells, bacteria, and virus) from small amount of diverse clinical samples (e.g., tissues, blood, serum, and urine). The development of simple, reliable, and sensitive probes is a strong current scientific priority. [5,6]

Canthium parviflorum Lamk (Rubiaceae) is a shrubby and woody plant found throughout the Western Ghats. Members of Rubiaceae are distributed more widely all pans of the earth. All the genus of the family are economically important. Canthium is a genus of about 230 species of shrubs or small trees. Plant pacifies vitiated kapha, diarrhea, fever, leucorrhea, worm infestation and general debility. In siddha system of medicine the plant was used in respiratory disorder, diuretic, diabetic, obesity. In Ayurvedha system of medicine the plant was used in cough, diuretic, tumor and as anthelmintic. An antioxidant, wound healing activity and antitumor acitivity were reported. D-mannitol, phenolic acid, phenolic compounds, carbohydrates, proteins were found from Canthium parviflorum.(CP) Pharmacological activities such as antimicrobial, antioxidant, antidiabetic, wound healing, diuretic, anti-inflammatory, antinociceptive, antitumor and antipyretic from various species of Canthium has been reported. [7,8,9]

2.MATERIALS AND METHODS

2.1 Plant material and Preparation of Extracts and Fractions

Fresh plant materials of Canthium parviflorum Lamk leaves was collected from Tambaram, Chennai during the month of November & December 2009. were botanically identified and authenticated by Prof. Jayaraman, Plant Anatomy Research Centre, Tambaram, Chennai, Tamilnadu, India.

The shade dried Canthium parviflorum Lamk leaves were coarsely powdered and extracted with ethanol using soxhlet extraction apparatus until exhaustive extraction. The solvent was removed using rotary vacuum evaporator and solvent free extracts were subjected for column chromatography (silica gel 60-120) and further broad fractioned by successive solvents using hexane, chloroform, ethylacetate and methanol solvents. The different fractions collected were concentrated by rotary vacuum evaporator and subjected for in vitro antiarthritic, and antioxidant studies.

2.2 *In vitro* anti-arthritic activity by inhibition of protein denaturation method

Procedure

1. The Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0 .05ml of various extracts/fraction of both plants (250 µg/ml).
2. Test control solution (0.5ml) consist of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0 .05ml of distilled water.
3. Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution (250 µg/ml).
4. Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0.05ml 0f Diclofenac sodium (250 µg/ml). All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37°C for 20 min and the temperature was increased to keep the samples at 57°C for 3 min. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416nm. [10,11]

The percentage inhibition of protein denaturation can be calculated as,

Percentage inhibition = [100-(optical density of test solution – optical density of product control) ÷ (optical density of test control)] ×100.

The control represents 100% protein denaturation .The results were compared with Diclofenac sodium (250 µg/ml). The percentage inhibition of protein denaturation of different concentration was tabulated.

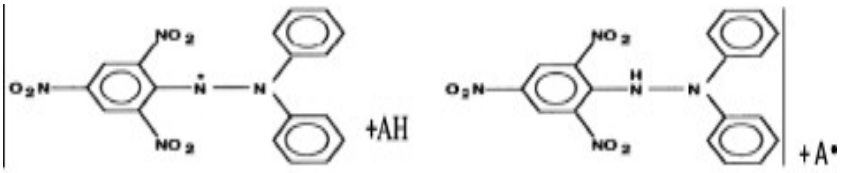
2.3 *In vitro* antioxidant activity

Antioxidant activity should not be concluded based on a single antioxidant test model. And in practice several in vitro test procedures are carried out for evaluating antioxidant activities with the samples of interest. Another aspect is that antioxidant test models vary in different respects. Therefore, it is difficult to compare fully one method to other one. Generally in vitro

antioxidant tests using free radical traps are relatively straight forward to perform. Among free radical scavenging methods, DPPH method is furthermore rapid, simple (i.e. not involved with many steps and reagents) and inexpensive in comparison to other test models. On the other hand ABTS decolorization assay is applicable for both hydrophilic and lipophilic antioxidants.

2.4 DPPH scavenging activity

The molecule 1, 1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color.



Diphenylpicrylhydrazyl (free radical) Diphenylpicrylhydrazine (nonradical)

In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored. The alcoholic extracts and their respective fractions of both plants in different concentration (0.2 ml) is diluted with methanol and 2 ml of DPPH solution (0.5 mM) is added. After 30 min, the absorbance is measured at 517 nm. The percentage of the DPPH radical scavenging is calculated using the equation as given below: $\%\ \text{inhibition of DPPH radical} = ([A_{br} - A_{ar}]/A_{br}) \times 100$ where A_{br} is the absorbance before reaction and A_{ar} is the absorbance after reaction has taken place.

2.5 Hydrogen peroxide scavenging (H_2O_2) assay: Human beings are exposed to H_2O_2 indirectly via the environment nearly about 0.28 mg/kg/day with intake mostly from leaf crops. Hydrogen peroxide may enter into the human body through inhalation of vapor or mist and through eye or skin contact. H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($OH^{\bullet}$) that can initiate lipid peroxidation and cause DNA damage in the body. The ability of plant extracts to scavenge hydrogen peroxide can be estimated. A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 mM pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm

using a spectrophotometer. The alcoholic extracts and their respective fractions of both the plants in different concentrations is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as follows: $\%\ \text{scavenged}\ (H_2O_2) = [(A_i - A_t)/A_i] \times 100$ where A_i is the absorbance of control and A_t is the absorbance of test.

2.6 Scavenging of 2,2'-azino-bis (3-ethyl benzo thiazole-6-sulphuric acid) di ammonium salt (ABTS)radical cation: The working solution was prepared by mixing stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate in equal amounts and allowing them to react for 12 h at room temperature in the dark. The resulting solution was later diluted with distilled water, and the absorbance read at 734 nm using a UV–visible spectrophotometer. A total of 1 ml of freshly prepared ABTS solution was added to 1 ml of the alcoholic extracts and their respective different fractions of different concentrations, the reaction mixture was vortexed for 10 s and the absorbance was measured at 734 nm after 6 min.

2.7 Scavenging of Hydroxy Radical in the Para-Nitraso Di methyl aniline (p-NDA): To the solution containing ferric chloride (0.1mM, 0.5ml) ascorbic acid (0.1ml of 0.5ml) H_2O_2 (2mM of 0.5ml) pNDA (0.01mM of 0.5ml) in phosphate buffer (pH 7.44, 20mM) were added with the various concentrations of the extracts and their respective different fractions of the plants or standards in distilled DMSO (0.5ml) to produce a final volume of 3ml. Absorbance was measured at 440nm. [12,13]

RESULTS

In vitro antiarthritis

Table 1 Effect of extracts and fractions of Canthium parviflorum on inhibition of protein denaturation

S.No	Extracts/fractions/Standard(250µg/ml)		% percentage inhibition
1	Extract	EME	95.42
2	Extract	ECP	82.57
3	Fractions	HFCP	13.23
4		CFCP	47.78
5		EAFCP	68.45
6		MFCP	78.56
7	Standard	Diclofenac sodium	98.36

Concentrations of extracts/fractions were taken 250µg/ml for all the sample.

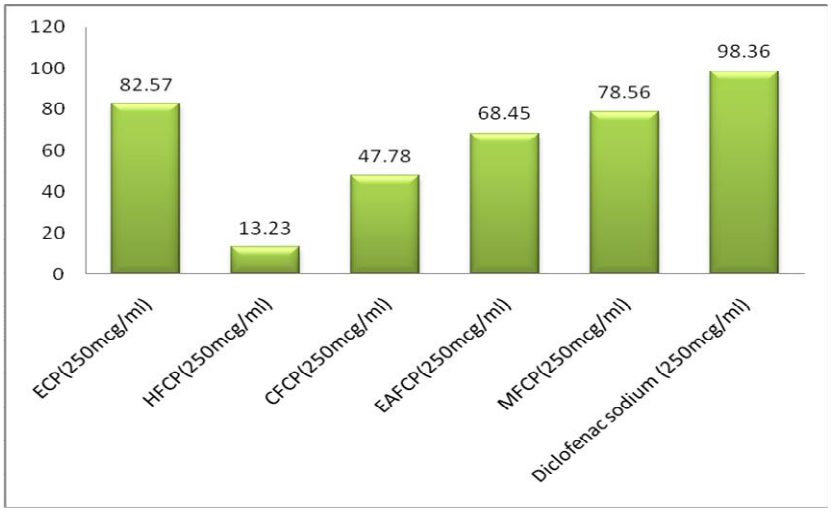


Fig 1 Effect of extracts and fractions of Canthium parviflorum on inhibition of protein denaturation

***In vitro* antioxidant activity**

Table 2: Effect of extracts and fractions of Canthium parviflorum on DPPH , H_20_2, ABTS, p-NDA

S.No	Extracts/Fractions/ Standard		IC_{50} Values in(µg/ml) ±SEM			
			DPPH	H_20_2	ABTS	p-NDA
1	Extract	EME	26.5 ± 1.2	77.6 ± 1.45	14.6 ± 0.95	>100
6	Extract	ECP	37.6 ± 0.34	45.8 ± 0.67	25.7 ± 1.56	>100
7	Fractions	HFCP	95.7 ± 1.2	>100	48.9 ± 0.67	>100
8		CFCP	87.9 ± 0.78	>100	59.9 ± 1.23	>100
9		EAFCP	65.4 ± 0.98	65.7 ± 1.13	35.8 ± 0.57	>100
10		MFCP	35.4 ± 0.56	54.4 ± 1.67	20.9 ± 1.45	>100
11	Standard	Ascorbic acid	12.3 ± 0.98	57.6 ± 0.57	18.5 ± 0.68	>100
12		Rutin	0.89 ± 0.07	1.25 ± 0.79	12.7 ± 1.18	-
13		Butylated hydroxyanisole BHA	-	18.57 ± 1.09	-	-

The IC_{50} values were determined 3 times, ±SEM, The potent antioxidant activity of extracts/fractions were determined by minimal IC_{50} with different model.

The preliminary in vitro screening was carried out to find out the active extracts and fractions of Canthium parviflorum for further proceeding of biological studies.

The results of in vitro antiarthritis studies revealed that the percentage inhibition of protein denaturation was significantly exhibited by alcoholic extract of CP (82.57) and methanol

fraction (78.56) of CP. The percentage inhibitions more than 70% were taken for biological studies.

The in vitro antioxidant activity was already reported for the extracts of CP exhibited significant activity with different methods. The results of the above studies showed significant antioxidant activity for alcoholic extracts of CP and also with ethyl acetate and methanol fractions CP. In the DPPH method, the alcoholic extracts, ethyl acetate and methanol fraction of CP showed potent antioxidant activity, with IC_{50} values ranging from 20-60 µg/ml. However, the standards rutin and ascorbic acid exhibited better results with lower IC_{50} values. In the H_20_2 method, the alcoholic extracts and methanol fraction showed potent activity which is comparable with the standard. In the ABTS method, all the extracts and fractions of CP exhibited potent antioxidant activity. In the p-NDA method, the alcoholic extracts and methanol fractions of CP exhibited antioxidant activity. All the extracts and fractions of CP exhibited moderate or potent antioxidant activity.

4. DISCUSSIONS

Most of the investigators have reported that denaturation of protein is one of the cause of rheumatoid arthritis. The production of auto antigen in certain arthritic disease may be due to in vivo denaturation of protein. The mechanism of denaturation propably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. From the above results the plants extracts/fractions which showed significant activity were selected for in vivo biological studies. [11]

Free radical and reactive oxygen species are well known inducers of cellular and tissue pathogenesis leading to several human diseases, such as cancer, inflammatory disorders, rheumatoid arthritis, and diabetes mellitus, as well as in the aging process. Many plant species with antioxidant activities act as protective agents against these diseases. The potent antioxidant activity was observed using many methods for all Extract and fractions of CP plants. From the above results, The CP plant extract and fractions exhibited potent antioxidant activities.

From the above in vitro studies the alcoholic extracts of CP and ethyl acetate and methanol fraction of CP were selected for phytochemical and biological studies to know the phytoconstituents responsible for the above activity.

5. CONCLUSION

The above invitro studies concluded that extracts and fractions showed moderate or potent antioxidant and anti arthritis activity. Further these extracts and fractions of Canthium parviflorum were used for invivo activity

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