

INVITRO ANALYSIS OF ANTI-INFLAMMATORY ACTIVITY OF CINNAMOMUM TAMALA AND OINTMENT FORMULATION

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Abstract: Even before modern medicine, spices were appreciated for their ability to help people fight infection and sustain good health. Cinnamomum Tamala (Family: Lauraceae) has been used in the treatment of inflammation for centuries. Anti-inflammatory activity means a medicine or substance with anti-inflammatory action lowers inflammation (redness, swelling, and pain) in the body. Anti-inflammatory drugs work by preventing the body from producing particular compounds that cause inflammation. They're used to cure a variety of ailments. The goal of this study was to invent an anti-inflammatory ointment that prevent the inflammation. Invitro anti-inflammatory activity of Cinnamomum Tamala extract (0.1-1ml) was studied by protein denaturation method. Dichloro phenac sodium was taken as standard drug on concentration(10µg-100µg). The findings suggest that the anti-inflammatory ointment by using Cinnamomum Tamala has substantial anti-inflammatory properties and may be useful in the treatment of inflammatory illnesses.

Keywords: Cinnamomum Tamala, Anti-inflammatory activity, anti-inflammatory ointment

1.INTRODUCTION

India is a plant-based country that is also known for its spices. Spices have always played an important part in exploration and growth. People are becoming more interested in spices these days, not just to improve the flavour of their food, but also because of the accumulated evidence in complementary and alternative medicine. Thanks to ongoing research and accumulating data, spices' medicinal advantages are becoming more well-known. A diet high in spices, fruits, and vegetables has been linked to a 20% reduction in all cancers in preventative medicine. Spices are also used in soap and toothpaste, as well as in the cosmetic and perfumery sectors. Spice essential oils are also used in ayurveda and allopathic preparations. Cinnamomum is one of the oldest spices, with a sweet aroma and a warming flavour. Cinnamomum Tamala belongs to the Lauraceae family of plants. Indian bay leaf and Tejpat are two local names for it. Cinnamomum is a genus that has been widely used to treat a variety of ailments. Cinnamomum Tamala has been reported to help with digestion and appetite stimulation. Cinnamomum Tamala also have the following properties antidiarrheic, antitumor, anti-inflammatory, anti-arthritic, antiparasitic, gastro urinary, antitumor, antiparasitic, antioxidant, chemo preventive, and gastroprotective. This plant grows best in a hot, humid climate. The plant is commercially grown in several parts of the country. Cinnamomum Tamala leaf output and essential oil are used for commercial purposes in India. Cinnamomum Tamala is a plant that contains several active components. Cinnamaldehyde, eugenol, and linalool were discovered in cinnamon bark oils. Chemoprotective action has been documented for several substances. In ancient times, Sudarshan choorna and Chandraprabhavati were two formulations made from this plant. It also has pharmacological qualities that can be used to treat and prevent a variety of ailments. Cinnamomum Tamala also having a very good anti-inflammatory activity, so by making use of this property an anti-inflammatory ointment was formulated and through the invitro method of testing the study confirms that the ointment have anti-inflammatory property. I have done the protein denaturation method to prove that the plant Cinnamomum Tamala have an good anti-inflammatory activity on comparing with the synthetic drug that I have taken as standard.



Fig no:1 cinnamomum Tamala (indian bay leaf)

1.1 PHYTOCHEMICAL CONSTITUENTS

Furanosesquiterpenoids are one of the main ingredients in the leaf essential oils of these species. Furanogermerone (59.5%) was discovered to be the most abundant chemical in the leaf essential oil, followed by caryophyllene (6.6%), sabinene (4.8%), germacrene D (4.6%), and curcumenol (4.6%). 2.3 percent The leaf oil had a high amount of sesquiterpenoids (96.8%), dominated by furanosesquiterpenoids (79.3%), namely furanodienone (46.6%), curzerenone (17.6%), furanodiene (1.8%), and curzerene (1.8%). 1.2 percent Cinnamon leaf oil has a number of components, including eugenol and cinnamaldehyde, which are mucous and

skin membrane irritants in the surrounding area. According to phytochemical analysis, polyphenols, flavonoids, alkaloids, flavones, flavonols, tannins, carbohydrates, amino acids, proteins, saponins, and glycosides were found in the leaves of *Cinnamomum tamala*, according to phytochemical analysis.



Fig no : 2 cinnamomum Tamala

2. MATERIALS AND METHOD

2.1 COLLECTION OF SAMPLE

Dried Leaves of *Cinnamomum Tamala* were collected from the spices shop from Palakkad district of Kerala.

2.2 PREPARATION OF SAMPLE

Leaf sample of *Cinnamomum Tamala* were collected. The dried leaves were crushed, and the milled using a laboratory blender into fine powder. The fine powder of the *Cinnamomum Tamala* leaf were taken for further analysis. The powder of *Cinnamomum tamala* looks olive green in colour.

2.3 PHYTOCHEMICAL SCREENING

Phytochemical examination were carried out as per the standard methods. The preliminary phytochemical analysis were carried out by using the leaf extract of *Cinnamomum Tamala* followed by the presence of alkaloids were identified by Mayer's and Wagner's test. Phenols were identified by ferric chloride test. Foams test results the presence of saponins and Molisch test for carbohydrates. Detection of flavonoids done by lead acetate test. Amino acids were identified by using ninhydrin test and sterols were identified by Libermann-Burchard test.

2.4 GC-MS ANALYSIS

Gas Chromatography-Mass Spectrometry (GCMS), is a combination of gas chromatography and mass spectrometry used to identify unknown samples and separate volatile and semi-volatile compounds. Gas chromatography-Spectrometer analysis was done by using flame ionisation detector. Hydrogen was used as fuel gas and nitrogen as carrier gas. GC consist of a capillary column HP-5 (5% Phenyl Methyl Siloxane) Capillary (30mX320umX0.25um). The carrier gas used was nitrogen at 2.5 ml/min flow rate. 1 micro litre sample was injected by autoinjector. The temperature of injector 260°C and detector was set at 240°C. The primary temperature of column is 100°C raised to 220°C for 3 min, again raised temperature 260°C for 5 min. Whole process of GC carried out at 50 mins temperature 260°C at 50°C/min.

2.5 ANTIOXIDANT ACTIVITY

Antioxidant activity of dried bay leaf was done through determination of antioxidant by DPPH assay. Antioxidants inhibit lipid oxidation, so scavenging of DPPH (Diphenyl-1-picrylhydrazyl) radical hence determine free radical scavenging ability. It is widely used method. 0.1 mM solutions of DPPH in methanol prepared, 1ml of this solution added into 3 ml extract in methanol (concentrations 50, 100, 200, 400 & 800 micro gram/ml). shaken vigorously allowed to stand for 30 minutes at room temperature. Read absorbance at 517 nm. If the absorbance value is lower, the more is the free radical scavenging capacity. The capacity of radical scavenging calculated by following formula;

$$\text{DPPH scavenging effect (\% of inhibition)} = \left\{ \frac{(A_b - A_s)}{A_b} \right\} * 100$$

Where, A_b is absorbance of control reaction, A_s is absorbance in presence of all extract sample and reference. All tests performed in three times and results were averaged.

2.6 OINTMENT PREPARATION

Wound healing ointment is prepared as per the procedure of simple ointment. Simple ointment is prepared by fusion method. simple ointment contains wool fat, hard paraffin, ceto- steryl alcohol and white soft paraffin. The white soft paraffin, is the base for simple ointment, in which wool fat was added as a non emulsifying absorption base. Wool fat have an absorption capacity of more than 50%. Hard paraffin is added to stiffen the preparation and to maintain the consistency. Hard paraffin was melted in a chinadish and wool fat ceto steryl alcohol and white soft paraffin was added. The ingredients are heated in a water bath until it get melted and fused together. The mixture was cooled and stirred well until a semi-solid mass is formed.



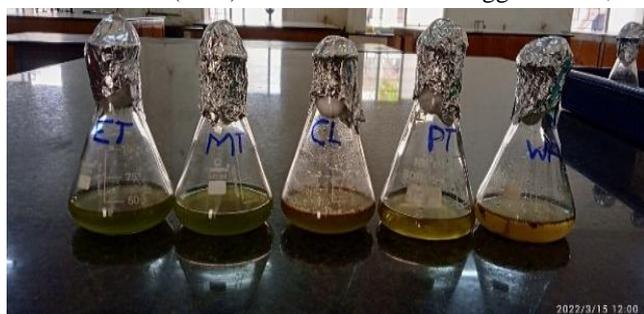
Fig no :3 ointment base preparation

2.7 ANTI-INFLAMMATORY ACTIVITY

Denaturation of proteins Many of the weak connections, or bonds (e.g., hydrogen bonds), inside a protein molecule that are responsible for the highly organised structure of the protein in its natural (native) state are broken during denaturation. The structure of denatured proteins is looser and more random, and most of them are insoluble.

•To see if the polyherbal formulations are capable of preventing protein denaturation. The standard for this experiment is dichlorophenac sodium. It's an anti-inflammatory medication that can keep proteins from becoming denatured.

In vitro anti-inflammatory activity was conducted by protein denaturation method given by Mizushima and Kobayashi (1968). The reaction mixture (5 ml) contained 0.2 ml of egg albumin, 2.8 ml of phosphate-buffered saline (PBS, pH 6.4), and 2 ml of plant



extract at various concentrations (10, 20, 50, 100 ug/L). As a control, a similar volume of double distilled water is employed. The mixture was then heated at 70 °C for 5 minutes after being incubated at 37 °C for 15 minutes in a BOD incubator. After cooling, their absorbance was measured at 660 nm by using the vehicle as blank. The percentage of inhibition of protein denaturation was calculated.

• percentage of inhibition of protein denaturation was calculated by using the following formula:

$$\text{Inhibition} = \frac{\text{Ac}(660\text{nm}) - \text{At}(660\text{nm})}{\text{Ac}(660\text{nm})} \times 100$$

• AC (Absorbance of control solution), AT (Absorbance of the test

sample).

2.8 ANTIMICROBIAL ACTIVITY

The Well Diffusion technique was used to test the antibacterial and antifungal activities of crude extract extracts. 20ml of molten media was poured into sterilised petriplates to make MHA plates. After the media had solidified, a 20-25 l suspension of bacterial inoculums was uniformly swabbed. After dipping the sterile paper discs in the necessary solvents, they were placed in agar plates. The wells were then filled with 10-50 l of plant extract. The plates were then incubated for 24 hours at 37°C. Triplicates of the assay were run, and control plates were kept as well. The zone of inhibition was measured in millimetres from the well's edge to the zone. On mullerhintonagar plate and potato dextrose agar, the tested cell suspension was distributed. Using sterile forceps, wells were placed in the agar medium. Wells were poured with plant extract. The plates were then incubated at 37°C for around 24 hours, while the control was kept. The inhibition zone was measured in millimetres from the clear zone. Antibacterial activity was performed by agar diffusion method. The stock culture of bacteria(*E.coli*) were received by inoculating in nutrient broth media and grown at 37 °C for 18 hours. The agar plates of the above media were prepared. Each plates was inoculated with 18 hours old cultures the bacteria were swab in the sterile plates. Cut the 5 wells Pour the extract in ratio 25 µl, 50 µl, 75 µl, 100 µl. All the plates were incubated at 37°C for 24 hours and the diameter of inhibition zone was noted in Cm.

Agar well diffusion method has been used to determine the antimicrobial activities and minimum inhibitory concentrations or plant extracts against Gram positive, Gramnegative bacteria. The extracts exhibited antibacterial activities against tested microorganisms

3.RESULT AND DISCUSSION

3.1 PHYTOCHEMICAL SCREENING

The phytochemical analysis of the plant bay leaf was carried out and experiments show the presences of phytochemicals in ethanol extract contain flavonoids, carbohydrates. Ethanol extract give negative result for alkaloids, saponin, tannin, sterol, amino acid. Methanol extract showed positive result in saponin, flavonoids, sterol, carbohydrates. Methanol extract give negative in alkaloids, tannin, amino acid. Chloroform extract showed positive result for saponin, sterol, carbohydrates and negative in alkaloids, tannin, flavonoids, amino acid. Petroleum ether extract showed positive result for alkaloids, saponin, sterol, carbohydrates, amino acid. petroleum ether extract give negative in tannin, flavonoids. water extract gives positive result in alkaloids , flavonoids and shows negative result tannin , amino acid. Chloroform extract showed positive result for saponin, sterol, carbohydrates and negative in alkaloids, tannin, flavonoids, amino acid. Petroleum ether extract showed positive result for alkaloids, saponin, sterol, carbohydrates, amino acid. petroleum ether extract give negative in tannin, flavonoids. water extract gives positive result in alkaloids, flavonoids and shows negative result in saponin, tannin, sterol, carbohydrates, amino acid. **Showed in table 6.**

Fig no : 4 phytochemical solvent extraction
3.2 GC-MS ANALYSIS
GC-MS INTERPRETATION

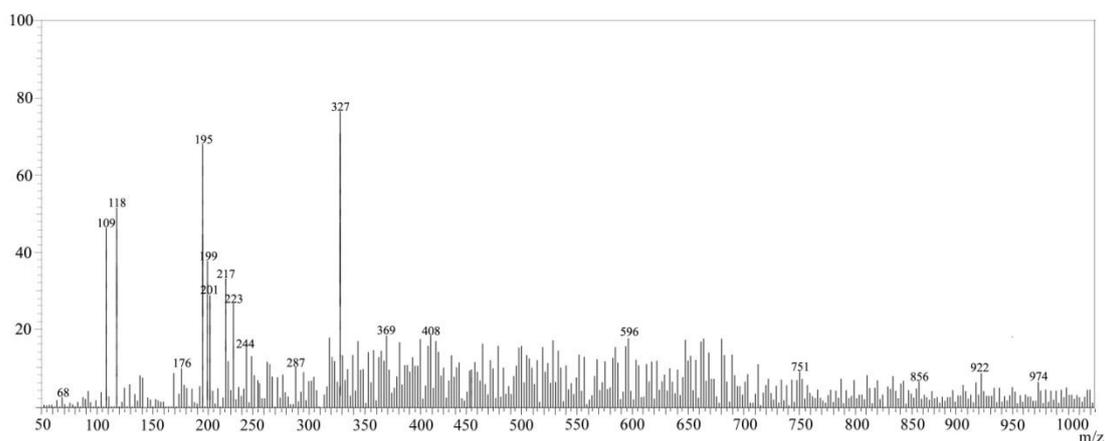


Fig no: 5 Graphical representation of GC-MS Analysis
x-axis retention time , y- axis absorbance

GC-MS Aanalysis

Table 1 GC-MS Analysis

SL NO	COMPOUND NAME	Content as per integrated peak area method as %
1	COUMARIC ACID	2.20
2	COUMARIN	3.45
3	ELLAGIC ACID,	8.80
4	QUERCETIN	1.24
5	QUERCETIN METHYL ETHER	9.08
6	3-METHYL ELLAGIC ACID	0.50
7	NARINGENIN	0.42
8	STEARIC ACID	2.20
9	PALMITIC ACID	3.30

Usually, x-axis of GC-MS chromatogram shows amount of time taken for analytes pass through column, and reach mass spectrometer detector. The peaks that are shown corresponds to the time at which each of components reached the detector. The y-axis or area of peaks, is a reflection of amount of analyte. When looking at GC-MS chromatogram, area will be based on the number of counts taken by mass spectrometer detector at retention point. unknown compounds are identified based on retention times of known standards with other detectors. The mass spectrometer then allows identification of compound by mass spectrum obtained at time of testing. In this GC-MS graph shows peak value for the compound quercetin methyl ether it's a plant pigment (flavonoid) has antioxidant and anti-inflammatory properties. The second peak value shows by the compound ellagic acid it act as an antioxidant and can decrease levels of inflammation to protect against disease.

. Showed in table 1.

3.3 ANTIOXIDANT ACTIVITY

Table 2 Antioxidant – DPPH activity of cinnamomum Tamala

Sample concentration (µg/ml)	Sample (OD value)	Ascorbic acid(% scavenging inhibition)	Cinnamomum Tamala (% scavenging inhibition)
20	0.112	0.18	37.777
40	0.118	0.27	56.296
60	0.160	0.38	57.894
80	0.172	0.45	61.777
100	0.195	0.56	65.178

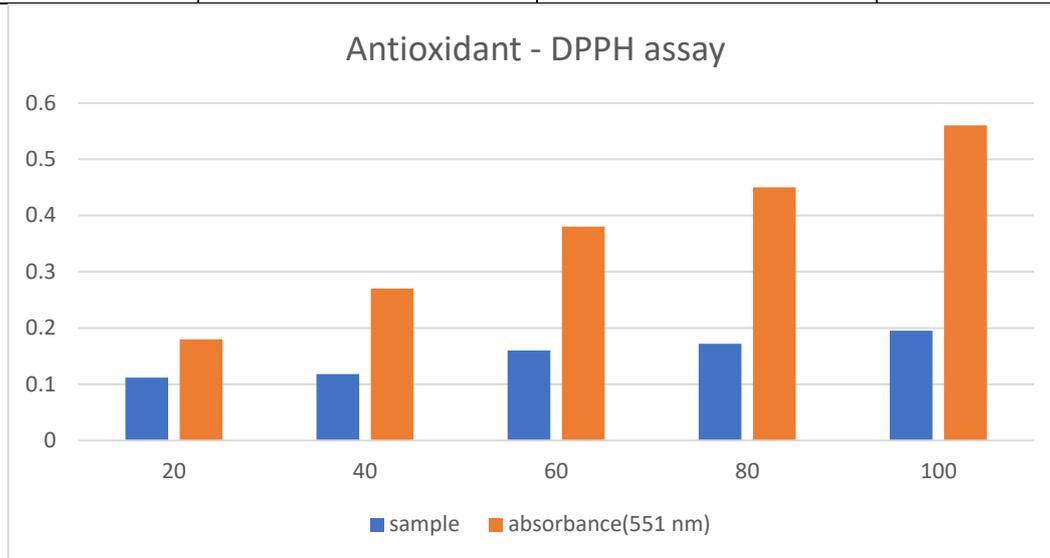


Fig no :6 x- axis concentration of ascorbic acid , y-axis absorbance

Antioxidant react with DPPH, stable free radical which is reduced to DPPH-H. The degree of discoloration indicates the scavenging potential of the antioxidant compounds. The study reveals that ethanolic extract of Cinnamomum Tamala serve as a good antioxidants.

3.4 FORMULATION OF OINTMENT

The present study was done to prepare and evaluate the herbal ointment. From this the herbal extract were prepared by using simple maceration process to obtain a good yield of extract and were was no any harm to the chemical constituents and their activity. The livagation method was used to prepare ointment so that uniform mixing of the herbal extract with the ointment base was occurred which was stable during the storage.



Fig no : 7 formulated ointment

Finally I made herbal ointment which can be very useful for preventing inflammation. Many people love the natural organic products which helps to naturally cures wounds , heal the skin and keeps our skin healthy.

Table 3 Physiochemical evaluation of ointment

PHYSIOCHEMICAL PARAMETERS	OBSERVATIONS
Colour	Olive green
Odour	Characteristic
Consistency	Smooth
pH	5.4
Spreadability	16.42
Extrudability	0.6gm
Diffusion study	0.8
Loss of drying	23%
Solubility	Soluble in boiling water
Washability	Good
Non irritancy	Non irritant
Stability study	Stable
Viscosity	4.320

3.5 ANTI-INFLAMMATORY ACTIVITY**Table 4 Reading from UV Spectrophotometer 3000” (LAB INDIA)**

Concentration in µg/ml	Standard	Cinnamomum Tamala	% of inhibition
10	0.064	0.044	31.25
20	0.741	0.562	24.15
60	0.889	0.652	26.65
80	1.772	0.932	47.40
100	2.212	1.482	33.00

The protein denaturation method was used to conduct the in vitro anti-inflammatory experiment. When compared to hexane, acetone, and chloroform extracts, the ethanolic extract had a high percentage of solubility. As a reference standard, dichlofenac sodium was used. The table shows the 560 nm absorbance of protein treated with the assay. Ointment shows higher anti-inflammatory activity on 80µg/ml. So the C.tamala ointment ethanolic extract demonstrated a high percentage of inhibition.

3.6 ANTIMICROBIAL ACTIVITY**Table 5 Antimicrobial activity of Cinnamomum Tamala**

Organisms	<i>S.aureus</i>	<i>E.Coli</i>
25 µl	0.4 cm	0.3 cm
50 µl	0.5 cm	0.4 cm
75 µl	0.6 cm	0.5 cm
100 µl	0.7 cm	0.8 cm
Standard (Chloramphenicol)	0.7 cm	0.7 cm



Fig no: 7 **Fig no: 8**
1.Staphylococcus aureus **2. E-coli**

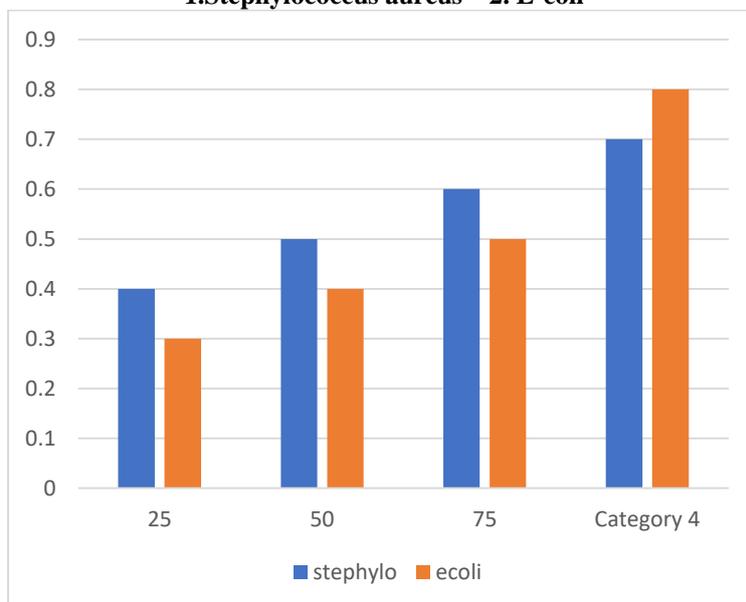


Fig no : 9 Graphical representation of anti-microbial activity
x-axis organisms , y- axis zone of inhibition

The graph represents the x-axis as quantity of organisms and y-axis as diameter of zone of inhibition. It shows zone of inhibition of methanolic extract of *Cinnamomum tamala* against staphylococcus aureus and E.coli. More antimicrobial activity shows in 100µl of concentration . C.tamala ointment shows more anti-inflammatory activity against E.coli bacteria on comparing with staphylococcus aureus bacteria

Phytochemical screening result

Table 6 phytochemical screening analysis for positive and negative results of the secondary metabolites present in

TEST	ETHANOL	METHANOL	CHLOROFORM	PETROLEUM ETHER	WATER
ALKALOIDS (Mayer's and wagner's test)	-	-	-	++	++
SAPONINS (foam's test)	-	+++	++	+	-
TANNINS (gelatin test)	-	-	-	-	-
FLAVANOIDS (lead acetate)	++	+++	-	-	++
STEROL (libermann burchard test)	-	++	+++	++	-
CARBOHYDRATES (Molisch test)	+++	+++	++	++	-
AMINO ACID (ninhydrin test)	-	-	-	++	-
PHENOL (ferric chloride test)	+	++	++	-	-
GLYCOSIDES (legal test)	++	+	-	++	
Diterpenes (copper acetate)	+	++	-	-	++

Cinnamomum Tamala(leaf).

3.7 CONCLUSION

Bay leaf is a naturally available spice that can be used to make an anti-inflammatory ointment. The plant contains the compounds that are responsible for anti-inflammatory activity. The anti-inflammatory ointment made with Cinnamomum Tamala has the ability to prevent inflammation that occurs due to wounds, cuts or by some microbial attack. The findings suggest that Cinnamomum Tamala has substantial anti-inflammatory properties and may be useful in the treatment of inflammatory illnesses.

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