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FT-IR ANALYSIS AND IN VITRO ANTI-INFLAMMATORY ACTIVITY OF LEAF EXTRACT OF MICHELIA CHAMPACA (L.)

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ABSTRACT

The present study is to investigate the Phytochemical, FT-IR and invitro anti- inflammatory activity of ethanol leaf extract of Michelia champaca. The preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, saponins, tannins, steroids, triterpenoids, coumarins, glycoside, and reducing sugar. FT-IR spectrum confirmed the presence of (O-H str), (C=C str), (C-H str), (C=C str), (C-H bond), (C-N str), (C-O str), (C≡C-H: C-H bond) groups. The anti-inflammatory activity was evaluated using albumin denaturation assay and membrane stabilization at different concentrations (200, 400, 600, 800, 1000 µg/ml). The in vitro anti-inflammatory activity of the extract was concentration dependent, with the increasing concentration, the activity is also increased. Aspirin was used as standard drug. The percentage of inhibition was compared with those of standard drugs. The maximum percentage inhibition was observed at a dose of 1000µg/ml. From the results, it is concluded that secondary metabolites present in the leaf extract responsible for the anti-inflammatory activity.

KEYWORDS: Michelia champaca, anti-inflammatory activity, albumin denaturation, membrane stabilization, aspirin.

INTRODUCTION

The inflammatory process is the response to an injurious stimulus. It can be evoked by a wide variety of noxious agents (e.g., infections, antibodies or physical injuries). The ability to mount an inflammatory response is essential for survival in the face of environment pathogens and injury; in some situations and diseases, the inflammatory response may be

exaggerated and sustained without apparent benefit and with severe adverse consequences (Hardman et al., 2001).

The inflammatory response dilutes elements or destroys the agents causing it and starts the sequence of events that heal and reconstitute the damaged tissue (Kumar et al., 2004). In the absence of inflammation, wounds and infections would never heal and progressive destruction of tissue would compromise the survival of the organism. Regarding bad side, it can lead to life threatening anaphylactic response to insects bite or reptiles, drugs, toxins, etc. and chronic diseases such as rheumatoid arthritis, atherosclerosis, fibrous bands and adhesions in intestinal obstruction (Harsh Mohan et al., 2002). Symptoms of inflammation include: (i) Redness (Rubor) due to gross and persistent dilatation of arterioles, capillaries and venules in the injured area; (ii) Swelling (Tumors) due to increased permeability of small blood vessels which allows the exudates to escape into the tissues of the damaged area; (iii) Heat (Calor) due to considerable increase the in blood flow; (iv) Pain (Dolar) due to release of certain endogenous chemical substances such as bradykinin, 5-HT (Serotonin) and certain prostaglandins (Kumar et al., 2004).

Michelia champaca L. (Magnoliaceae) commonly known as svarna champa, a tall handsome tree with yellow fragrant blossoms, is commonly used by many traditional herbal preparations. The plant is also reported to have significant wound healing (Dwajani and Shanbhag, 2009), antimicrobial (Khan et al., 2002), antidiabetic (Jarald et al., 2008) antitumor (Hoffmann and Torrance, 1977), anti-inflammatory (Vimala et al., 1977) antioxidant (Hasan et al., 2009) and antiinfective (Oumadevi, 2011) properties. The present study demonstrates the FT-IR analysis and Invitro anti-inflammatory activity of Michelia champaca leaves by inhibition of albumin denaturation and HRBC Membrane stabilization activity.

MATERIALS AND METHODS

Collection of plant materials

The fresh leaves of Michelia champaca linn were collected from Mannargudi, Thiruvarur district, Tamilnadu, India. The collected materials were cleaned, shade dried and coarsely powdered. The plant material was powdered and used for further studies.

Preparation of ethanol extract

The dried leaves are powdered using grinder, 100 gm of the powdered leaves was packed evenly in the soxhlet extractor and subjected to extraction with 500ml ethanol. After extraction, the solvent was distilled off and the extracts were concentrated on water bath to a dry residue and kept in a dessicator. The crude extract was used for further studies.

Qualitative analysis of phytochemical and screening

Ethanolic extract of Michelia champaca leaves are subjected to preliminary screening of phytochemical constituents. The procedures were analyzed qualitatively by the method of Sofowara (1193) and Harbrone, (1973).

FT-IR (Fourier Transform Infrared Spectroscopy)

Fourier transform infrared spectroscopy is a photochemical analytical technique that does not resolve the concentration of individual metabolites but provides a snapshot of the metabolic composition of a tissue at a given time. FT-IR can be employed to determine the structure of unknown composition and in the intensity of the absorption spectra associated with molecular composition or content of the chemical group. The FT-IR method measures the vibration of bonds within chemical functional group and generates a spectrum that can be regarded as a biochemical or metabolic "fingerprint" of the sample. By attaining IR spectra from plant samples, it might possible to detect the minor changes of primary and secondary metabolite (Mc cann et al., 1992).

INVITRO ANTIINFLAMMATORY ACTIVITY

Inhibition of albumin denaturation

The assay was done followed by the method of Mizushima et al, (1968) was followed with minor modifications. The reaction mixture consisted of extracts at different concentrations (200-1000µg/ml) and 1% aqueous solution of bovine albumin fraction. The pH of the reaction mixture was adjusted to 6.5 using 1N HCl and incubated at 37oC for 20 min and then heated at 57 ºC for 30 min. The denaturation process is stopped by cooling the samples and finally the turbidity was measured using spectrophotometer at 660 nm. Aspirin was used as the reference standard and the control was taken without the extract. The denaturation of protein inhibition by the extract and standard were expressed as percentage by using the formula, Percentage of inhibition = (Control – Test)/Control x 100.

Membrane stabilization assay

Preparation of Red Blood cells (RBCs) suspension (Sakat et al., 2010; Sadique, et al.,1989) The Blood was collected from healthy human volunteer who has not taken any NSAIDs (Non Steroidal Anti-Inflammatory Drugs) for 2 weeks prior to the experiment and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline.

Heat induced haemolysis (Sakat et al., 2010; Shinde,1999)

The reaction mixture (2ml) consisted of 1 ml test sample of different concentrations (200 - 1000 µg/ml) and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 ºC for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples.

The Percentage inhibition of Haemolysis was calculated as follows:

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

RESULTS AND DISCUSSION

The present study carried out the Michelia champaca L. leaf extract revealed the presence of medicinally active metabolites are summarized in the Table 1. The phytochemical evaluation of the leaf extract showed the presence of alkaloids, flavonoids, phenols, saponins, tannins, steroids, triterpenoids, coumarins, glycoside, and reducing sugar and absence of quinones, and phlobtannins.

Table 1: Qualitative Phytochemical Analysis of Michelia champaca leaves

S.No	Phytoconstituents	Results
1	Alakaloids	+
2	Flavonoids	+
3	Saponins	+
4	Quinones	-
5	Tannins	+
6	Steroids/Triterpenoids	+
7	Phenols	+
8	Coumarins	+
9	Glycosides	+

10	Phlobtannins	-
11	Reducing Sugar	+

Plants are important source of functional components for the development of new chemotherapeutic agents. Phytochemical investigation of the ethanolic extracts of Tylophora revealed the presence of various phytochemicals such as phenolic compounds, flavonoids, saponins, steroids, tannins. Phytomedicine have been used for the treatment of diseases as in done in cases of Unani and Ayrvedic system of medicines, a natural blueprint for the development of new drug (Viji et al., 2010).

The FT-IR spectrum was used to identify the functional group of the active compounds based on the peak value on the region of infrared radiation. The ethanol extract of Michelia champaca leaves passed into the FT-IR and the functional group of the compounds were separated based on its peak ratio. The results of FT-IR analysis confirmed the presence of alcoholand (O-H str), (O-H str) carboxylic acid, (C=C str), alkynes, (C=C str) alkanes, (C-H band)alkanes, (C-H str) aromatics, (C-N str) aromatic amines, (C-O str) alcohol, carboxylic acid, ester, ethers, (C≡C-H:C-H bond) alkynes which shows major peaks at 3465.88, 3432.45, 2981.17, 2905.28, 2093.84, 1639.19, 1451.14, 1402.56, 1267.53, 1077.39, 1046.05, 877.40, and 671.20cm^{-1} respectively Table 2 and Figure 1.

Table 2: FT-IR peack values of ethanolic extract of Michelia champaca leaves

S.No	Group frequency Cm^{-1} of the sample	Bond	Functional groups
1	3465.88	O-H Stretch	Alcohol, Phenol
2	3432.45	O-H Stretch	Alcohol, Phenol
3	2981.17	O-H Stretch	Carboxylic acid
4	2905.28	O-H Stretch	Carboxylic acid
5	2093.84	C=C Stretch	Alkynes
6	1639.19	C=C Stretch	Alkanes
7	1451.14	C-H band	Alkanes
8	1402.56	C-N Stretch	Aromatics
9	1267.53	C-O stretch	Aromatic amines
10	1077.39	C-O stretch	Alcohol, Carboxylic acids, ester, ether
11	1046.0	C-H Stretch	Alcohol, Carboxylic acids, ester, ether
12	877.40	C-H Stetch	Aromatics
13	671.20	C≡C-H:C-H bond	Alkynes

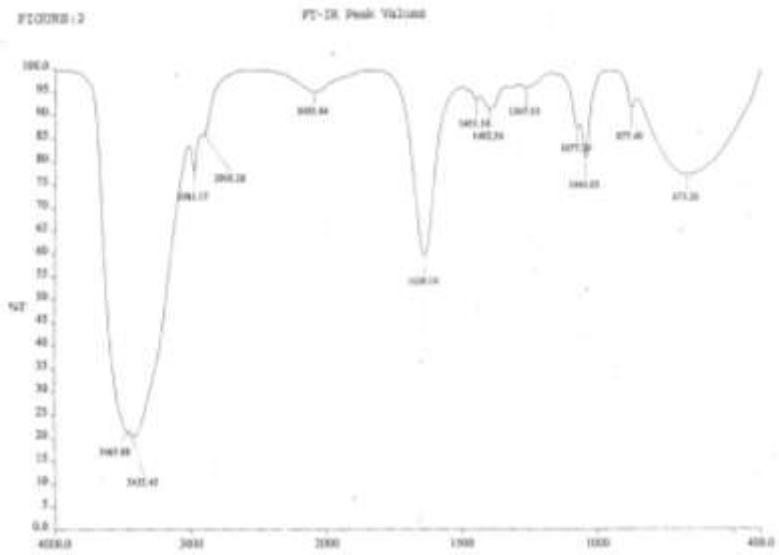

Figure 1: FT-IR spectra of ethanolic extract of Michelia champaca leaves

The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FT-IR spectrum confirmed the presence of alcohol, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in different extracts. In addition, UV-VIS and FT-IR spectroscopy is proved to be a reliable and sensitive method for detection of bio molecular composition (Komal Kumar et al., 2009).

InvitroAnti-Inflammatory Activity

Albumin Denaturation

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied.

It was showed from the result of the present study, it can be stated that the ethanol leaf extract of M.champaca is capable of inhibiting denaturantion of proteins. Maximum inhibition of 87% was observed at 1000 µg/ml. Aspirin, a standard anti-inflammatory drug showed the maximum inhibition of 80% at the concentration of 200µg/ml. The results are summarized in Table 3.

Table 3: Invitro anti inflammatory activity of ethanolic extract of Michelia champaca leaves and aspirin of albumin denaturation method.

Treatments	Concentration (µg/ml)	Absorbance at 560nnm	% inhibition of albumin Denaturation
Control	-	6.33 ± 0.12	-
M.C	200	1.64 ±0.04	74
M.C	400	1.33 ±0.04	78
M.C	600	1.12 ±0.02	82
M.C	800	0.95 ± 0.15	84
M.C	1000	0.79 ±0.03	87
Aspirin	200	1.2 ±0.01	80

Values are expressed as Mean ± S.D

Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation (Grant et al., 1970). Denaturation of protein is a well document cause of inflammation in condition like Rheumatoid arthritis. These protein against protein denaturation, which was the main mechanism of action of NSAIDs postulated before the discovery of their inhibitory effect of cyclooxygenase by Vane, (1971) may play an important role in the anti rheumatic activity of NSAID.

HRBC Membrane Stabilization Method

In the study of membrane stabilization activity of Michelia chmapaca at concentration range of 200, 400, 600, 800, 1000 µg/ ml protected significantly in a concentration dependent manner the erythrocyte membrane against lysis induced by hypotonic solution.

It showed the maximum inhibition of 99% at a concentration of 1000 µg/ml with the increasing concentration. The membrane hemolysis is decreased and membrane stabilization/ protection are increased. Hence anti-inflammatory activity of the extract was concentration dependent. In this study, the extract was effective in inhibiting the heat induced hemolysis at different concentrations as shown in **Table 4**.

Table 4: Effect of Michelia champacaon ethanolic extract of HRBC Membrane Stabilization Method.

Treatments	Concentration (µg/ml)	Absorbance At 560 nm	% inhibition of hemolysis
Control	-	2.71 ± 0.15	-
M.C	200	0.77 ± 0.02	71
M.C	400	0.58 ± 0.05	78
M.C	600	0.46 ± 0.15	83

M.C	800	0.32 ± 0.01	88
M.C	1000	0.02 ± 0.05	99
Aspirin	200	0.51 ± 0.01	81

Values are expressed as Mean ± S.D

During inflammation, lysosomal hydrolytic enzymes are released which causes damages of the surrounding organelles and tissues with attendance variety of disorders. The erythrocyte membrane is analogous to the lysosomal membrane (Chou et al., 1997) and its stabilization implies that the extract may as well stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage upon extracellular release (Murugasan et al., 1981).

CONCLUSION

In conclusion the results indicate that the ethanolic leaf extract of Michelia champaca possess anti-inflammatory activities. These activities may be due to the presence of alkaloids, flavnoids, triterpenoids, coumarins, glycosides, reducing sugar. For further study may lead to the development of a potential drug that may treat various kinds of infection and may lead to full utilization by the local communication.

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