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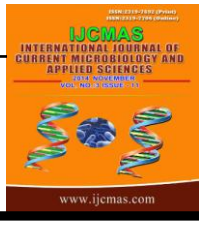

Original Research Article

Quantitative Estimation of Phytochemicals and assay of *in-vitro* Anti-inflammatory activity of White Ekka (*Calotropis gigantea*) using Albumin Denaturation Method

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Keywords

White ekka, *Calotropis gigantea*, Anti-inflammatory, Albumin denaturation

A B S T R A C T

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed drugs in the world today for the treatment of inflammatory conditions. On the other hand, NSAID side effects like gastrointestinal irritation occur with prolonged use. Consequently, there is a growing interest in discovering novel natural remedies and anti-inflammatory medications. Thus, present study was conducted with the main purpose of quantitative estimation of phytochemicals and assay of *in-vitro* anti-inflammatory activity of aq. leaf extract of White ekka (*Calotropis gigantea*) using albumin denaturation method. Results revealed that total phenolic content of aq. leaf extract of White ekka was found to be highest (1.148 ± 0.21 GAE, mg/100 mg) as compared total flavonoids (0.88 GAE, mg/100 mg). There was a dose dependent inhibition (%) was observed in standard as well as aq. leaf extract of White ekka. Furthermore, the inhibition (%) aq. leaf extract of White ekka at the concentration of 750 µg/mL was comparable with that of standard drug i.e., Aspirin. While, at the concentration of 1000 µg/mL inhibition (%) aq. leaf extract of White ekka was better than that of standard drug i.e., Aspirin. In conclusion, results of our study clearly demonstrated that aq. leaf extract of White ekka possess anti-inflammatory activity. Therefore, it could be recommended that aq. leaf extract of White ekka could be employed for the management of inflammatory conditions and could be considered for development of natural anti-inflammatory drugs.

Introduction

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants (Ferrero-Miliani *et al.*, 2007). The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Proinflammatory

cytokines (e.g., tumor necrosis factor - a [TNF-a], interleukin [IL-6], and IL-1b) are produced in large quantities by activated macrophages/monocytes that stimulate cellular responses via increasing prostaglandins (PGs) and reactive oxygen species (ROS).

In addition, lipid peroxidation (malondialdehyde [MDA]) is produced by free radicals attacking the cell membranes. Thus, inflammatory effect results in the accumulation of MDA (Janero, 1990).

Recent studies with a number of herbal extracts have shown promising results. It has been shown that these compounds isolated from various medicinal plants express their anti-inflammatory activities by down regulating expression of several crucial proinflammatory mediators like inducible NO synthase (iNOS), PGs, interleukin-1 β (IL-1β), TNF-α and IL-10. (Nirmal *et al.*, 2012; Devi *et al.*, 2003; Singh and Majumdar, 1995) Due to the adverse effects of nonsteroidal anti-inflammatory drugs and opioids, the search is on for new drugs with lesser side effects. Many valuable drugs of today (e.g., atropine, ephedrine, tubocurarine, digoxin, reserpine, aspirin, vincristine, morphine, and quinidine) came into use through the study of herbal and indigenous remedies. (Singh *et al.*, 2010)

Calotropis gigantea belongs to the family *Apocynaceae*. The family has a worldwide distribution in tropical and warm climates and is found abundantly in tropical forests (Figure 1).

It is commonly called as “crown flower” or “giant milk weed” is a well-known weed to many cultures for treating various disorders related to central nervous system, skin diseases, digestive system, respiratory system, reproductive system, etc. (Kadiyala *et al.*, 2013). It is used as a traditional medicinal plant and is used to treat common disease such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, and diarrhea. According to Ayurveda, dried whole plant is a good tonic, expectorant, depurative, and anthelmintic (Kumar *et al.*, 2010). The leaves are useful in the treatment of paralysis, arthralgia, swellings, and intermittent fevers.

The leaves have been found to have sedative and anxiolytic effect. (Khan Nalwaya *et al.*, 2009) The traditional practitioners use the leaf extract for the treatment of inflammatory painful conditions and rheumatic pain.

With this background, the present study was conducted with the main purpose of quantitative estimation of phytochemicals and assay of *in-vitro* anti-inflammatory activity of aqueous (aq.) White ekka (*Calotropis gigantea*) using albumin denaturation method.

Materials and Methods

Collection of plant material

The leaves of White ekka were collected in and around Chikkaballapura district, Karnataka, India. The leaves were sprayed with ethanol, and then shade dried at room temperature for 10 days. The dried leaves were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Extraction

Approximately 50 g of dried and coarsely powdered leaves of White ekka were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 550

mL of double distilled water. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The extracts were preserved in airtight containers and stored at room temperature until further use.

Estimation of total phenolic content

The total phenolic content in aq. leaf of White ekka was determined by the modified method of Folin-Ciocalteu method as described by Singleton *et al.,* (1999). Briefly, 500 µL of different concentrations of extracts was mixed with 0.5 mL of 10-fold diluted Folin-Ciocalteure agent.

After 5 min 0.5ml of 7.5% Sodium carbonate solution, 4.5ml of double distilled water were added, vortexed and incubated in a dark placefor120min, the optical density was measured at 760 nm against a blank. The total phenolic content was calculated on the basis of the calibration curve of gallic acid standards (10ppm-100 ppm) and expressed as gallic acid equivalents (GAE), in milligrams per 100grams of the sample (mg/100 mg sample).

Estimation of total flavonoid

Aluminum chloride colorimetric method was used for flavonoids determination in aq. Leaf extract of White ekka (Ordonez *et al.,* 2006). The flavonoid content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg gallic acid equivalent/100 mg of extract powder.

***In-vitro* anti-inflammatory activity assay**

The *in-vitro* anti-inflammatory activity of aq. leaf extract of White ekka was determined using modified method. Control, Standard (Aspirin), and different concentrations of aq. leaf extract of White ekka (i.e., 100-1000 µg/mL) were prepared as follows;

Control

2 mL of egg albumin, 28 mL of phosphate buffer (pH 6.4) and final volume was made up to 50 ml with double distilled water.

Standard (Aspirin)

2 ml of egg albumin, 28 mL of phosphate buffer (pH 6.4) and different concentrations (100-1000 µg/mL) of standard drug (Asprin) were taken and final volume was made up to 50 ml.

Extract

2 mL of egg albumin, 28 mL of phosphate buffer (pH 6.4) and different concentrations of aq. leaf extract of White ekka (i.e., 100-1000 µg/mL) were taken and final volume was made up to 50 ml.

The reaction mixtures of control, standard (Aspirin), and different concentrations of aq. leaf extract of White ekka (i.e., 100-1000 µg/mL) were incubated at 37ºC for 15 minutes and heated at 70ºC for 5 minutes.

After cooling, turbidity was measured at 660 nm. Percentage inhibition of albumin denaturation was calculated using the following formula. (Chandra *et al.,* 2012)

Inhibition (%) = $(1 - A2/A1)$ X 100

Where,

A1 = Absorption of the control sample

A2 = Absorption of the test sample

Results and Discussion

Total phenolic and flavonoid content

The results of total phenolic and flavonoid content of aq. leaf extract of White ekka was represented in Table 1 and plotted in Figure 2. Results revealed that total phenolic content of aq. leaf extract of White ekka was found to be highest (1.148 ± 0.21 GAE, mg/100 mg) as compared to total flavonoids (0.88 GAE, mg/100 mg).

The results of *in-vitro* anti-inflammatory activities of standard and aq. leaf extract of White ekka was presented in Table 2 and Figure 3. Results revealed that the mean inhibition (%) exhibited by standard was found to be 88.80, 121.02, 174.45, and 296.50 at the concentrations of 250 µg/mL, 500 µg/mL, 750 µg/mL, and 1000 µg/mL respectively. Similarly, the mean inhibition (%) exhibited by aq. leaf extract of White ekka at concentrations of 250 µg/mL, 500 µg/mL, 750 µg/mL, and 1000 µg/mL was found to be 48.43, 107.44, 218.20, and 444.21 respectively. These findings depicted that there was a dose dependent inhibition (%) was observed in standard as well as aq. leaf extract of White ekka.

Furthermore, the inhibition (%) *of* aq. leaf extract of White ekka at the concentration of 750 µg/mL was comparable with that of standard drug i.e., Aspirin. While, at the concentration of 1000 µg/mL inhibition (%) of aq. leaf extract of White ekka was better than that of standard drug i.e., Aspirin.

A number of factors, such as bacterial infection, chemical injury, and environmental pollution, can cause inflammation, which is a complicated process that can cause cell damage or death. The most widely used drugs in the world today are NSAIDs (O'Byrne and Dalgleish, 2001; O'Byrne and Dalgleish, 2000). The NSAIDs used to treat inflammatory conditions only alter the inflammatory response to the diseases, not the underlying cause of the disease. Market demand exists for orally active molecules that are more effective than currently available medications at treating the underlying causes of inflammatory disease as opposed to just the symptoms. Different methods such as inhibition of phosphatases, aminotransferases, cotton pellet granulation techniques, inhibition of heat-induced hemolysis, inhibition of albumin denaturation, membrane stabilizing, platelet aggregation, have been used to study the anti-inflammatory potentials of drugs or agents (Oyedapo *et al.*, 2010). With these viewpoints, the present study was conducted with the main purpose of assay of *in-vitro* anti-inflammatory activity of aq. leaf extract of White ekka using albumin denaturation method.

Denaturation of protein has an unpredictable mechanism which includes modification in electrostatic hydrogen, hydrophobic and disulfide bonding. (Kar *et al.*, 2012) Denaturation of protein causes the production of autoantigens in conditions such as rheumatic arthritis, cancer and diabetes which are conditions of inflammation. Hence, by inhibition of protein denaturation, inflammatory activity can be inhibited.

Concurrently, in our study, there was dose dependent inhibition (%) was observed in standard as well as aq. leaf extract of White ekka. Furthermore, the inhibition (%) *of* aq. leaf extract of White ekka at the concentration of 750 µg/mL was comparable with that of standard drug i.e., Aspirin. While, at the concentration of 1000 µg/mL aq. leaf extract of White ekka was better than that of standard drug i.e., Aspirin.

Table.1 Total phenolic and flavonoid content of aq. leaf extract of White ekka

Phytochemicals	Aq. Leaf Extract of White ekka (GAE, mg/100 mg extract)
Total phenol	1.148 ± 0.21
Total flavonoid	0.88 ± 0.18

Values were expressed Mean ± SD; n=3; GAE, Gallic acid equivalent

Table.2 Effect of aq. leaf extract of White ekka extracton *in-vitro* anti-inflammatory activity

Concentration (µg/mL)	Inhibition (%)	
	Standard	Aq. Leaf Extract of White ekka
250	88.80	48.43
500	121.02	107.44
750	174.45	218.20
1000	296.40	444.21

Values are expressed as Mean; n=3

Figure.1 Showing White ekka (*C. gigantea*) plant

Figure.2 Total phenolic and flavonoid content aq. leaf extract of White ekka

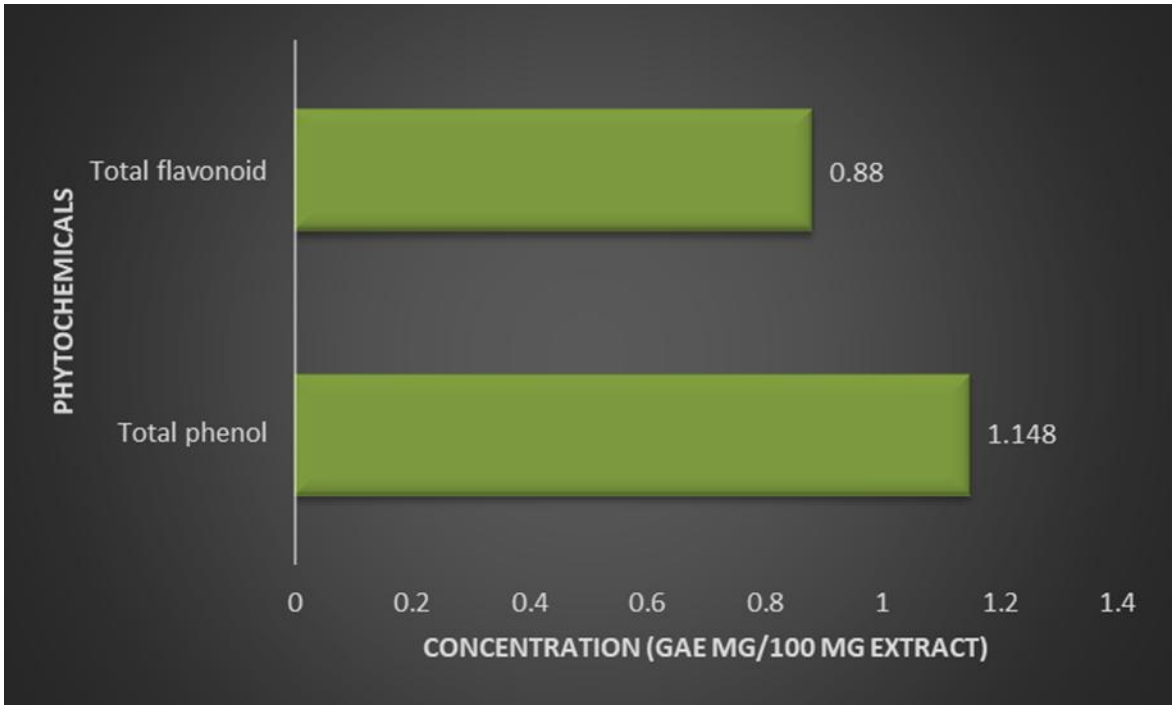
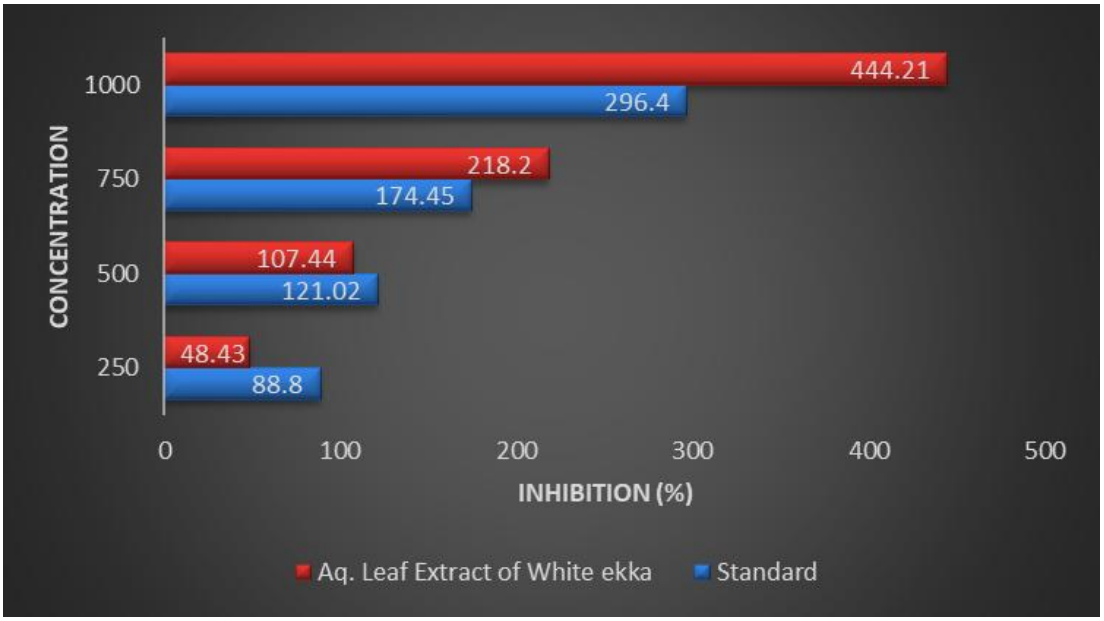


Figure.3 Effect of aq. leaf extract of White ekka on *in-vitro* anti-inflammatory activity



White ekka contains a galaxy of phytoconstituents which are thought to exert anti-inflammatory effect through a multi modal approach, hitting multiple therapeutic targets. Some of the possible targets reported for anti-inflammatory activity of natural products according to Choe, *et al.*, (2012) are as follows; Anti-inflammatory effect can be due to the inhibition and the stimulation of the production of cytokines

IL-12 and of IL-4, respectively, in addition to the decrease in NO; positive effect over proinflammatory markers, relieving oxidative stress and down regulating COX-2, TNFα, NF-κB, and IL-8; inhibition of the inflammatory cytokine induced production of PGE2 and NO which ultimately inhibits lipopolysaccharide-induced NO production in a dose dependent manner by the suppression of iNOS and COX-2 production and TNF-α and PGE2 inhibition (Shukla *et al.,* 2008).

The quantitative estimation of phytochemicals of aq. leaf extract of White ekka revealed considerable amount of total phenols and flavonoids, which have been duly credited for anti-inflammatory responses through multimodal approach.

Possible inhibition of COX synthesis in tandem with down regulation of NF-κB, scavenging of free radicals and inhibition of other proinflammatory mediators besides COX by down regulating their activity or gene expression could be the possible modes of action (Singh *et al.,* 2010). However, further studies at molecular level are required to elucidate the exact mechanistic pathway. However, this research shall form an excellent basis for developing the bioactive extract of the plant White ekka as an excellent drug candidate in the near future.

The results of present preliminary study clearly demonstrated that aq. leaf extract of White ekka possess anti-inflammatory activity.

Hence, it could be recommended that aq. leaf extract of White ekka could be employed for the management of inflammatory conditions and could be considered for development of natural anti-inflammatory drugs. However, further studies are recommended to elucidate the exact mechanism of action of particular phytochemical responsible for anti-inflammatory activity White ekka.

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