06.30.2025

JOURNAL OF

Health, Metabolism & Nutrition Studies (JHMNS) Vol. 8 No. 3

INVITRO ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF *Chrysanthenium indicum* EXTRACT

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DOI Link: https://doi.org/10.70382/bejhmns.v8i3.012

Abstract

The present investigation was aimed to justify the scientific basis in traditional use of chrysanthemum indicum as radicals scavenging herbal supplement. In-vitro antioxidant activities were evaluated by Reducing power method, Superoxide scavenging method and DPPH radical scavenging method. In-vitro anti-inflammatory activities were evaluated using albumin denaturation and membrane stabilizing method. Both methanol and aqueous extracts of Chrysanthenium indicum showed significant percentage inhibition of protein denaturation for methanol (61.87) and aqueous (51.14) extract compare to Diclofenac sodium (89.27); and hemolysis methanol (31.86) and aqueous (28.14) extract compare to Diclofenac sodium (51.27); at 0.10mg/ml. The extracts also exhibited antioxidant activities against ascorbic acid as a standard in which DPPH method for methanol (56.6) and aqueous (43.7) extract compare to ascorbic acid (85.7); super oxide radicals methanol (62.9) and aqueous (61.3) extract compare to ascorbic acid (85.8); and reducing power activity methanol (72.3) and aqueous (64.7) extract compare to ascorbic acid (87.3) at 100µg/ml The maximum

Introduction

Inflammation is the host response to trauma or as the defense mechanism against invasive organisms which eventually lead to redness, pain, swelling and temperature that evokes inflammatory cells (macrophages, neutrophils, monocytes, dendritic and mast cells) to invade the site of infection or wounds establishing an 'inflammatory microenvironment' that leads to the death and degradation on the organism, agent or affected cells and eventual restoration of cellular or organ repair process (Abeles & Solitair, 2008).

In many inflammatory disorders there is excessive activation of phagocytes, production of O_2-, OH radicals as well as non-free radical's species (H_2O_2) which can harm severely tissues either by powerful direct oxidizing

E-ISSN 3026-8664 P-ISSN3027-2238

96% inhibition by Gallic acid equivalents and Quercetin 81%. Total phenolic content equivalent flavonoids content in Methanol extracts were estimated that was three fold (phenolic content) is two fold (flavonoids) more than that in aqueous extract. These observations established the influence of solvent on both antioxidant and Invitro anti-inflammatory activities was obvious. Chrysanthemum indicum activities against inflammation and oxidative stress could be due to cyclooxygenase enzyme inhibition and free radical scavenging activities of the extract.

Keywords: inflammatory, antioxidant, free radicals, stress & disorder

action by activating matrix metalloproteinase damage seen in various arthritic tissues. Due to its implication in virtually all human and animal diseases, inflammation has become the focus of global scientific research, more so, since the currently used anti-inflammatory agents both steroidal and non-steroidal are prone to evoking serious adverse reactions. However, antioxidant supplements or antioxidant containing foods may be used to help the human body to reduce oxidative damage. Herbs and spices, in general are harmless sources for obtaining natural antioxidants. It is evident that there is an increasing demand to evaluate the antioxidant properties of direct plant extract. In spite development of new synthetic anti-inflammatory drugs, the search of new natural products is necessary from alternative sources. More than 40 million people worldwide are currently receiving synthetic Angiotensin Converting enzyme inhibitors which are associated with the development of adverse Consequences such as; Kidney failure, angioedema, hypotension, decrease in white blood cells etc. Several plants have been reported to have been used in the management of inflammation with no or less side effects. Chrysanthemum indicum has numerous medicinal applications and it is used traditionally for the treatment of inflammation among the local people Olajide et. al.,(2003). Recently it has been hypothesized that oxidative stress is a key player in the pathogenesis of human inflammation. Hence there is a need to simultaneously evaluate the antioxidant and anti-inflammation potency of aqueous and methanol extracts with the aim of identifying hypertensive drug candidate from plants. *Chrysanthemum indicum* of the family Asteracae is distributed widely in the northern part of Nigeria. The plant is reported to be used by different cultural groups for treatment of diarrhea, typhoid, cholera, chronic jaundice, fever, and headache and skin diseases. . Whole plant is anthelmintic, expectorant, tonic and used in the treatment of chest and skin diseases ethanomedically. The fronds have astringent properties and are found to strengthen and promote the repair of sinews, muscles and bones. They are effective for

lower back and ligament injuries C. indicum is used topically in traditional Chinese medicine to stimulate hair growth and to treat baldness. C. indicum along with other combination of herbs is used in pain from traumatic injury, such as sprains and contusions with bruising and swelling. With this background the present study designed with an aim to justify traditional claims of using C. indicum as source of anti-inflammatory agent with scientific tools (Abeles & Solitair, 2008). Chrysanthemum indicum has numerous medicinal applications and it is used traditionally for the treatment of inflammation among the local dwellers. The study is to investigate the effect of aqueous and methanol extracts of Chrysanthemum indicum on its anti-inflammatory and Antioxidant Properties. . Hence Inflammation is a risk factor for cardiovascular disease. It is the most common and persistent serious health problem; it affects 20-45% of the active population and carries a high risk.

The objectives of the present study are to; determine the phytochemical composition of the aqueous extracts and determine the antioxidant potency and inflammatory potentials of the methanolic and aqueous extracts.

Figure 1.0: *Chrysanthemum indicum* snapshot from Mubi, Adamawa state. Nigeria.

MATERIALS AND METHODS

MATERIALS

Equipment's /Apparatus used for this study are as Follows:
Beaker, Conical flask, measuring cylinder, weighing balance, Soxhlet extractor, Water bath, Test tubes, Syringe (1ml and 5ml), Grinder, Burette, Retort stand, Uvlight.

Chemicals, solvents and Reagents

Absolute ethanol, Tween-80, Ibuprofen, Benzene, Ammonia Potassium, mercuric iodide (mayers reagent), Glacial acetic acid, H2SO4 Hydrogen tetraoxosulphate (vi) acid, Ethyl acetate, Acetone, Chloroform, Ferric chloride.

METHODS

Plant Sample collection and identification

Plant was collected during the rainy season at Federal Polytechnic Mubi, North Local Government Area Adamawa State, Nigeria.
The Plant sample was identified and authenticated in Forestry Technology Department, Federal Polytechnic Mubi, Adamawa State.

Preparation of aqueous extract

The *Chrysanthemum indicum* was washed and air dried at room temperature. Dried samples were pulverized using pestle and mortar. Exactly one Liter of distilled water was added to 500g powdered leaves and soak for 24 hours. The filtrate was concentrated by evaporation using a water bath at 400C. After which the aqueous extract obtained was stored inside a container and keep until required at room temperature.

Phytochemical screening

Phytochemical screening for major constituents was undertaken using standard qualitative methods described by Odeyi and Sofowora (2008). Harbone 1976; Trease and Evans (2002). The plants extract were screened for the presence of glycoside, alkaloids, flavonoids, tannins, saponins and terpenoid, phenol, steroid, phlobatannin, carbohydrate and protein.

Quantitative determination of phytochemical constituents of the plant samples.

The method described by (Akimutini, 2006, Chang and Minga, 2002
Phenolic compound analysis was determined by spectrophotometric method
Determination of antioxidant activity by Karadag, A., Ozeelik, B. and Saner, S. (2009).(Halliwell 2010).

DPPH Free radical scavenging activity

The free radical scavenging activity of MECI and AECI were measured by 1,1-Diphenyl-2-picryl hydrazil using the method of Gulchin *et al.*, (2002).

Estimation of Total Phenolic Content

Total Phenolic content of extracts was determined by Folin– Ciocalteu method.

Estimation of Total Flavonoids

The total flavonoid content of the extracts was determined by aluminium chloride (AlCl3) colorimetric method..

IN-VITRO ANTI-INFLAMMATORY ACTIVITY

Methods of Mizushima and Kobayashi [1968] and Sakat *et al.*, (2010) followed with minor modifications.

MEMBRANE STABILIZATION TEST

Preparation of red blood cells [RBCs] suspension

Fresh whole human blood (10ml) was collected 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 reconstituted as 10% (v/v) suspension with normal saline.

Heat induced hemolysis

The reaction mixture (2ml) consisted of 1 ml of test sample solutions (MECI and AECI) at different concentration (200 – 800µg) and 1ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Diclofenac sodium (10mg) was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56^0C 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 5min and the absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicates for all the test samples.

Percentage of inhibition of hemolysis activity was calculated by the following formula,

% Inhibition = Control OD – Test OD X 100 Control OD

Where Control OD is the absorbance without sample, Test OD is the absorbance of sample extract / standard.

Result

Table 1: Preliminary Phytochemical screening of methanolic and aqueous extract of *Chrysanthenum indicum*

Sample	Alkaloid	Flavonoid	Phenol	Tanin	Steroid	Saponin	Protein	Carbohydrate	Reducing Sugar
MECI	-	+ +	+ +	+	+	-	+	+	-
AECI	-	+ +	+ +	+	-	-	+	+	-

Table 2: IC50 value of In-vitro antioxidant activities and total flavonoid and phenolic content of MECI and AECI.

S. No.	Sample	IC50 value (µg) of *In-vitro* antioxidant Activities			Total Flavonoids Content (100 µg of Quercetin equivalent)	Total Phenolics Content (100µg of Gallic acid equivalent)
		Reducing power activity	Super oxide anion scavenging activity	DPPH* scavenging activity		
1.	ASCORBIC ACID	87.3	85.8	85.7		
2.	MECI	72.3	62.9	56.6	81.03	95.972
3.	AECI	64.7	61.3	43.7	4.1	32.60

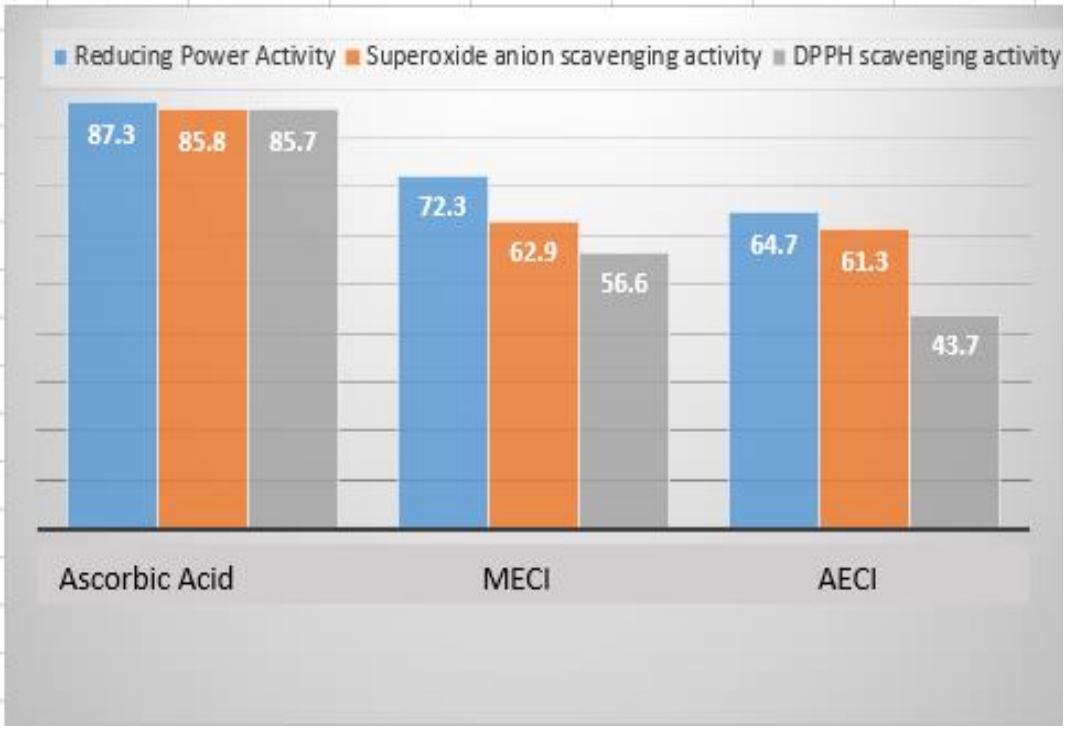

Fig 1: In-vitro antioxidant activities of MECI and AECI.

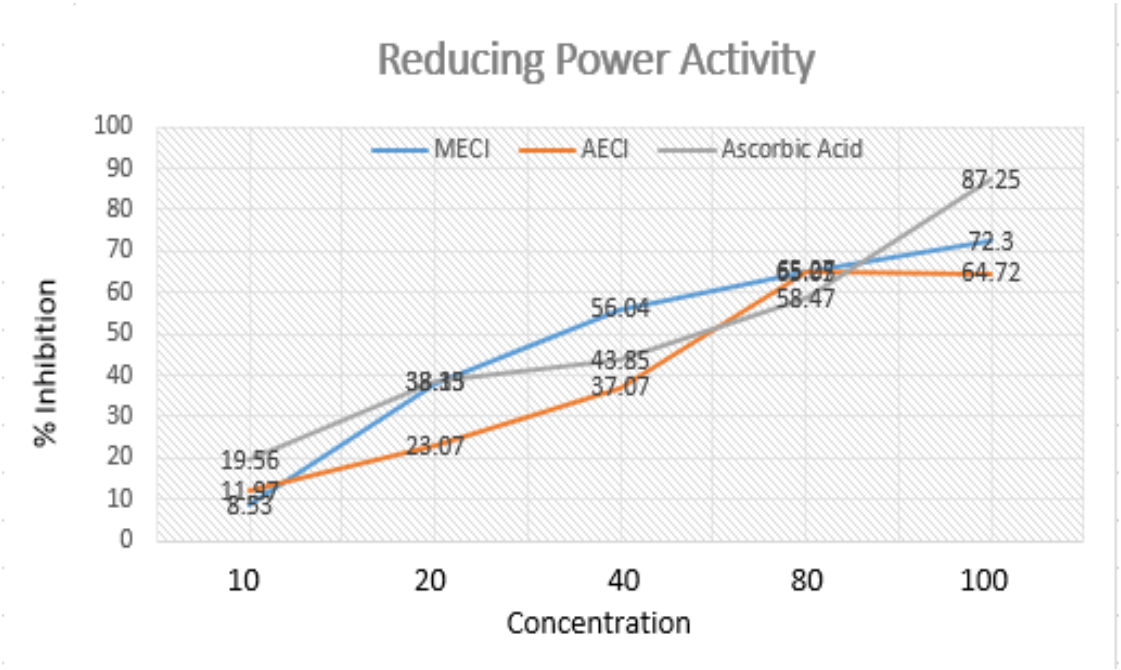

Fig 2: Reducing power activity in µg/ml

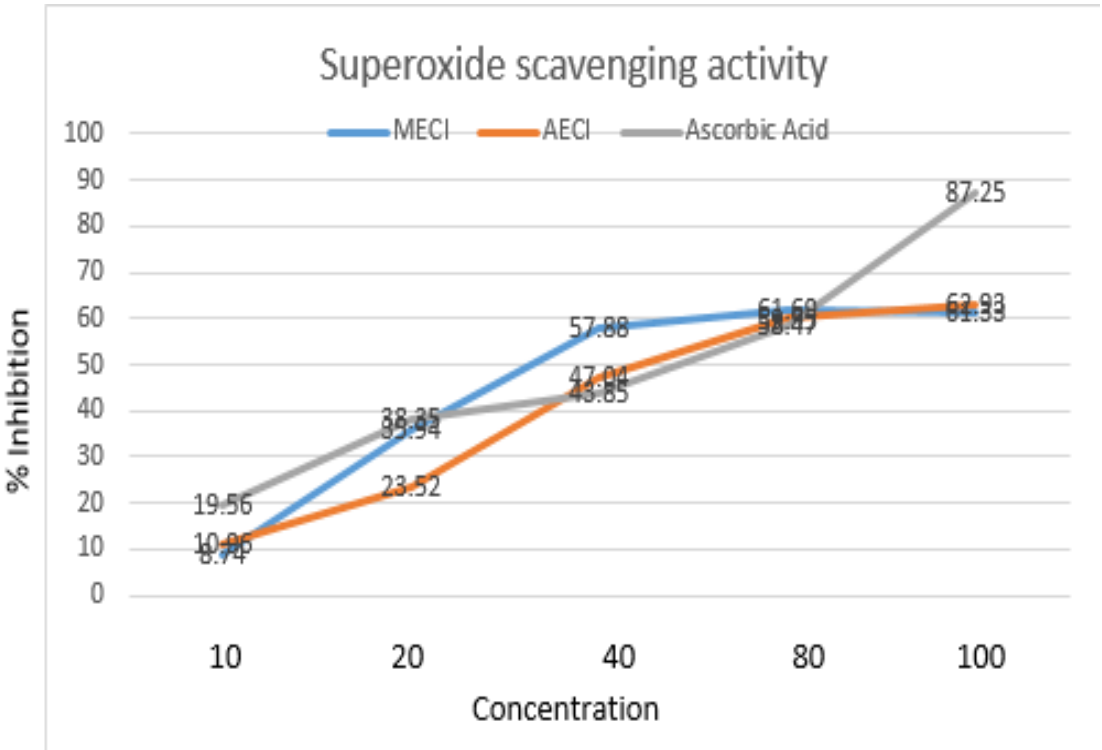

Fig 3: Superoxide scavenging activity in µg/ml

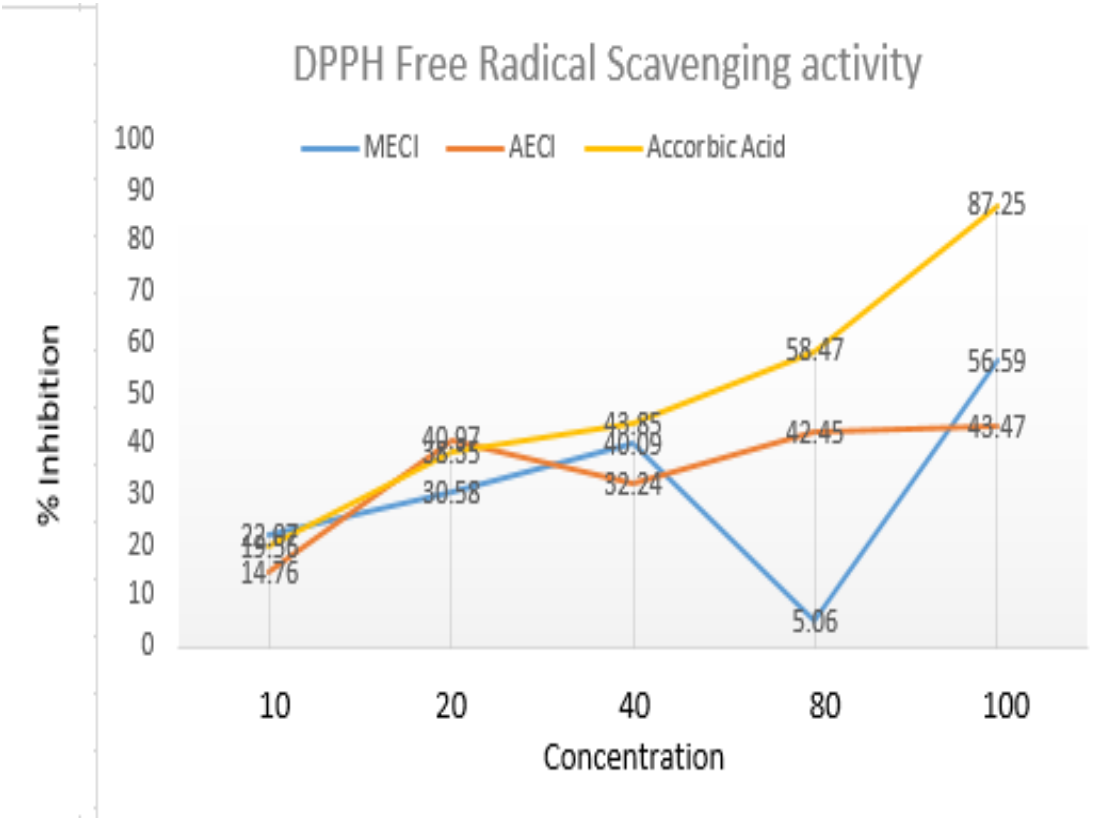

Fig 4: DPPH Free radical scavenging activity in µg/ml

Table 3: IC50 value of *In-vitro* anti-inflammatory of MECI and AECI

S. No.	Samples	IC50 Value (µg) of *In-Vitro* Anti-inflammatory activity	
		Albumin Denaturation Assay	Inhibition of Heat induced Hemolysis Assay
1	MECI	61.3	78.7
2	AECI	81.3	80.9
3	DIACLOFENAC SODIUM	51.5	35.6

The IC50 is Half maximal inhibitory concentration is a measure of the potency of a substance in inhibiting a specific biological or biochemical function. It indicates how much drug is needed to inhibit a biological process by half, thus providing a measure of potency of an antagonist drug in pharmacological research

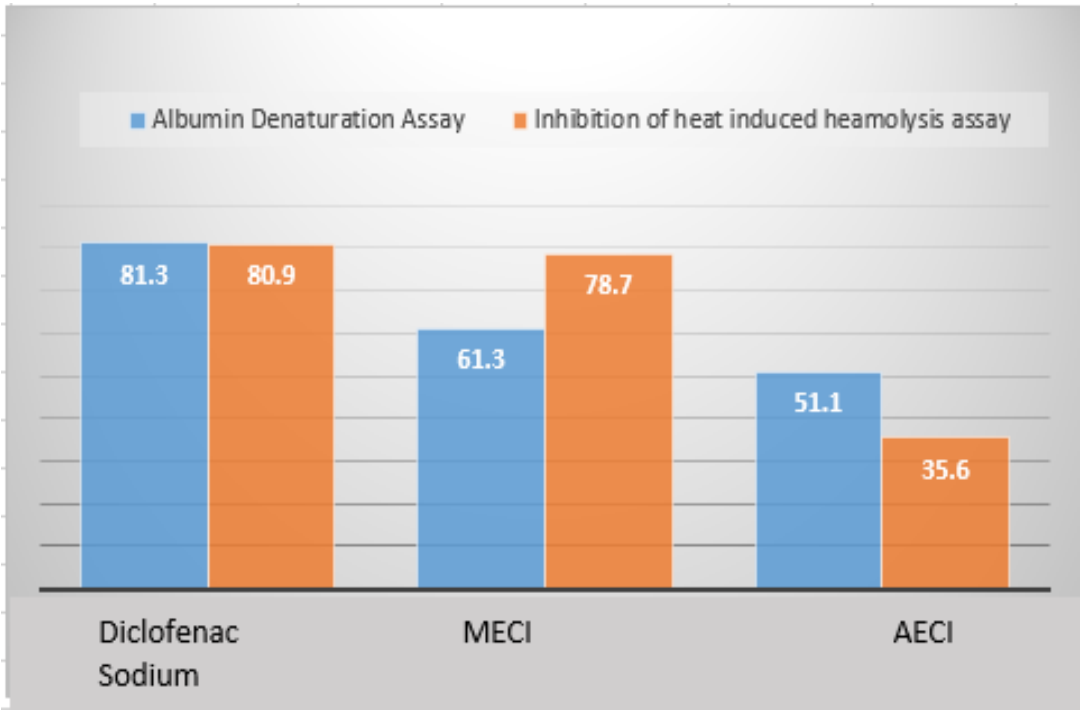

Fig 5: IC50 value of *In-vitro* anti-inflammation

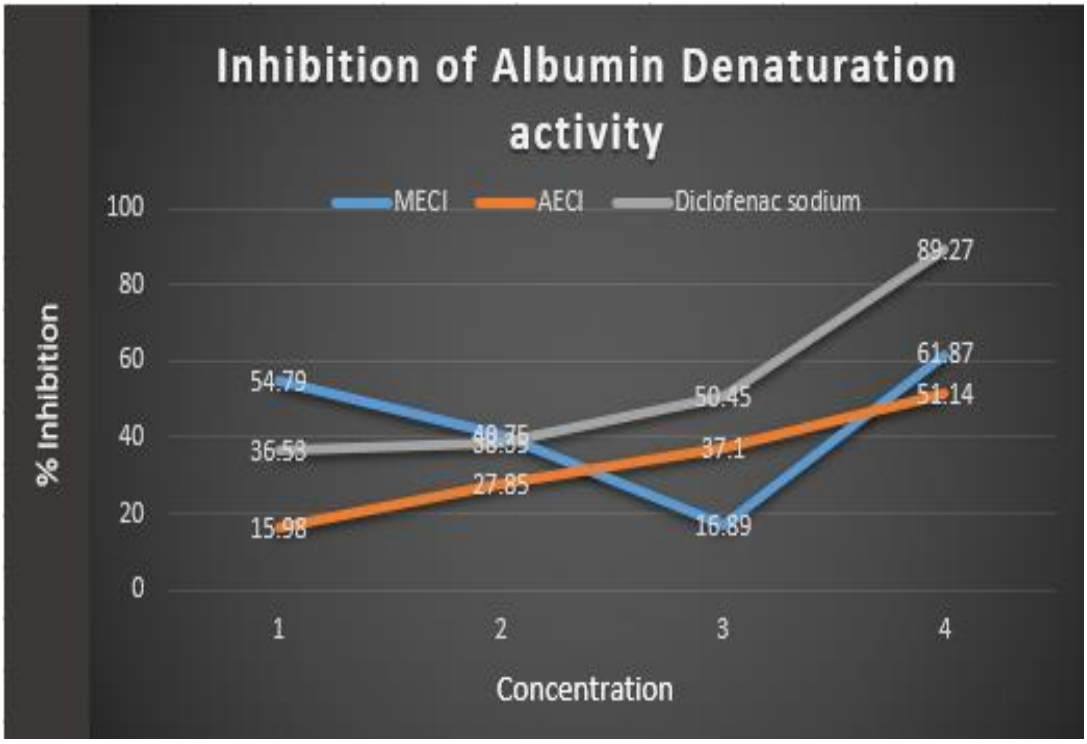

Fig 6: Inhibition of albumin denaturation activity in mg/ml

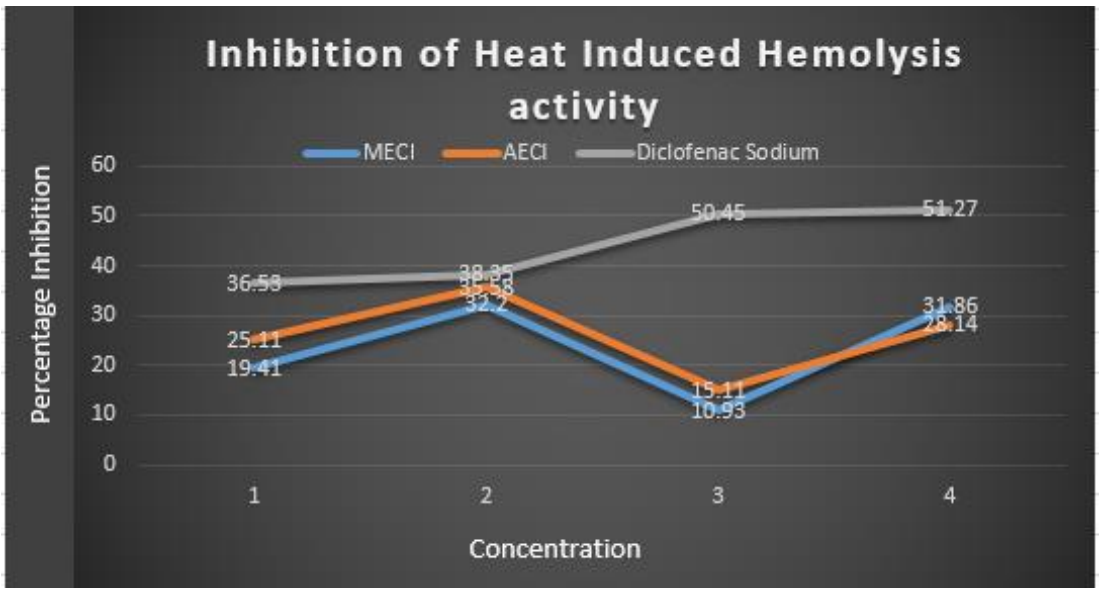

Fig 7: Inhibition of heat induced hemolysis activity in mg/ml

DISCUSSION

The present preliminary phytochemical study shows the presence of flavonoid, phenol, tannin, steroid, protein, carbohydrate in methanol extract and flavonoid, phenol, protein, carbohydrate in aqueous extract. Details of the result illustrated in Table 1 and 2. (Harborne1984).

The antioxidant potentiality of test samples (MECI and AECI) have been studied by different mechanisms, *viz*. prevention of chain initiation, binding of transition metal ion catalysts, reductive capacity and radical scavenging. Various antioxidant models and modifications have been proposed to evaluate antioxidant activity and to explain how antioxidants function, reducing power, superoxide scavenging assay, DPPH assay are most commonly used for the evaluation of antioxidant activities of extracts.

Figure 2, 3&4 shows the reducing power, superoxide and DPPH free radical scavenging capacity of MECI and AECI compared to standard ascorbic acid. For the measurements of the reducing power ability, we investigated the Fe3+ – Fe2+ transformation in the presence of MECI and AECI samples. Where, the reducing power of MECI and AECI showed similar activities to the control at different concentrations. The IC50 value was 72.3% for MECI and 64.7% for AECI, whereas; IC50 value of standard ascorbic acid was 87.3 % (Figure 1) similar to finding by Ali, G., Hawa, Z.E.J. and Asmah R. (2011).

In case of the NBT- superoxide anion system, MECI and AECI showed higher scavenging activities in dose dependent manner. Where, the decrease of absorbance at 700nm with antioxidants indicates the consumption of superoxide anions generated in the reaction mixture. The IC50 values of MECI (62.9%) shows highest superoxide scavenging activity

than AECI (61.3%) but their activity was less than standard ascorbic acid (85.8%) Karadag, A., Ozeelik, B. and Saner, S. (2009). Some herbs and health supplements have anti-inflammatory qualities. A cannabinoid also has anti-inflammatory effect (Dimarello, 1999). Oxidative stress occurs when free radical formation exceeds the body's capacity to protect itself and contributes different biological chronic conditions such as arteriosclerosis, arthritis, cancer, diabetes and various neurodegenerations. The primary antioxidants react with free radicals, which may limit free radical damage occurring in the human body. The observation of present study supported ability of MECI and AECI in protection against oxidative damage just similar to done by Jamuna, S., Subramanian, P. and Khrishnamoorthy, P. (2014)

DPPH in presence of ethanol produces stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Our study illustrates the hydrogen donating ability of MECI and AECI in various concentrations compare to standard antioxidant (L- ascorbic acid). The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compare to other methods. The reduction capability on the DPPH radical is determined by the decrease in its absorbance at 517nm induced by MECI and AECI when compare to control. The IC50 values of MECI (56.6%) shows highest superoxide scavenging activity than AECI (43.7%) but their activity was less than standard ascorbic acid (85.7%) (Figure 4). J.F Jr. and Vita, J.A. (2001). Flavonoids are a large class of compounds, ubiquitous in plants, and usually occurring as glycosides. They contain several polyphenols or phenol hydroxyl functions attached to ring structures. The cleavage of the glycosidic ring takes place possibly in the gastrointestinal tract releasing of the free polyphenols. The chemical activities of polyphenols components in terms of their reducing properties as hydrogen or electron donating agents predict their potential for action as free radical scavengers (antioxidants). Thus, free radical-scavenging activities of MECI and AECI may be attributed to the presence of flavonoids and other polyphenols in the extracts. Flavonoids and other plant phenolics are reported, in addition to their free radical scavenging activity having multiple biological activities including vasodilatory, anticarcinogenic, anti-50 inflammatory antibacterial, immune-stimulating, anti-allergic, antiviral, and estrogenic effects, as well as being inhibitors of phospholipase A2, and lipoxygenase (LOX), glutathione reductase and xanthine oxidase. These biological activities are related to their anti-oxidative effects. Ali, G., Hawa, Z.E.J. and Asmah R. (2011).

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 one

of the well-defined causes of inflammation. present study, AECI and MECI have shown inhibition of thermally induced protein (Albumin) denaturation in dose dependent manner, where the IC50 values of MECI (61.9%) shows highest inhibition of heat induced heamolysis than AECI (35.6%) but their activity was less than standard diclofenac sodium (81.3%) (Figure 5). Further, the HRBC membrane stabilization has been used as a method to study the *in-vitro* anti-inflammatory activity (Figure 7). because the erythrocyte membrane is analogous to the lysosomal membranes its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes release during inflammation produce a various disorder. The extra cellular activity of these enzymes are said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. The injury to RBC membrane will further render the cell more susceptible to secondary damage through free radical induced lipid peroxidation. It is therefore expected that compounds with membrane stabilizing properties, should offer significant protection of cell membrane against injurious substances. In present study, the IC50 values of AECI (32.2%) shows 51 highest inhibition of heat induced hemolysis than MECI (35.6%) but their activity was less than standard diclofenac sodium (80.9%) (Figure7). These extracts were effective in both heat induced albumin Denaturation and heat induces hemolysis indicated usefulness of test extracts against acute inflammation (Leelaprakash 2011) 52 (Dimarello, 2019).

In Conclusions based on the experimental analysis and result, the methanolic and aqueous extracts of C. indicum have shown the tendency to inhibit the *in vitro* activity of inflammation and it has been used in the past for the treatment of inflammation in the traditional medicine. Similarly, the various extracts demonstrated antioxidant activity that would be helpful in the management of oxidative stress. Recommendation from the study, we recommend that the methanolic and aqueous extracts of *C. indicum* possess *in vitro* antioxidant and anti-inflmmatory effects. But a work should be done on the *in vivo* inhibitory activity of methanolic and aqueous extracts of *C. indicum.*

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E-ISSN 3026-8664 P-ISSN3027-2238