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# In-vitro anti-inflammatory activity of organic extracts of stem of *Ziziphus jujube* Gaertn (L) var. *hysudrica* Edgew

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**Key words:** Stem, Extraction, Total phenolic content, Total flavonoid content, Anti-inflammatory activity

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## Abstract

Extracts of stem of *Zizyphus jujuba* (L.) Gaertn. Var. *hysudrica* Edgew were prepared by using methanol, acetone, ethylacetate, dichloromethane and chloroform. Their total phenolic contents and total flavonoid contents were determined by using single concentration of all extracts (800µg/ml). Total phenolic contents 35, 34.16, 2.44, 22.77 and 11.11mg eq GA/g were measured in acetone, methanol, dichloromethane, chloroform and ethylacetate extracts respectively. While total flavonoid contents 60.59, 57.96, 43.15, 39.53 and 38.22mg eq rutin/g were measured in acetone, ethylacetate, dichloromethane, methanol and chloroform extracts respectively. Anti-inflammatory activity of organic extracts of stem of *Zizyphus Jujuba* (L.) Gaertn. Var. *hysudrica* Edgew was measured by three methods; inhibition of albumin denaturation, anti-proteinase and RBC's membrane stabilization against heat induced and hypotonicity induced hemolysis assays. Acetone and methanol extracts have shown high anti-inflammatory activity 80% and 79.51% by inhibition of albumin denaturation and 90.92% and 89.57% by RBC's membrane stabilization against heat induced hemolysis and was related to phenolics present in these extracts. Dichloromethane and acetone extracts have shown high activity 96.70% and 96.16% by anti-proteinase assay and activity was attributed to both phenolics and flavonoids present in these extracts. Chloroform and dichloromethane extracts have shown high activity 72.04% and 71.49% by RBC's membrane stabilization against hypotonicity induced hemolysis assay due to synergistic effect of phenolics and flavonoids present in extracts. Data was statistically analyzed by using one way ANOVA. Multiple comparisons was performed by using LSD test, $p < 0.05$ was considered significant.

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**Introduction**

*Zizyphus Jujuba* (L.) Gaertn. Var. *hysudrica* Edgew is a hybrid of two Zizyphus species namely *Zizyphus mauritiana*and Zizyphus *spina-christi* (Azam-Ali *et al.*, 2006). It grows as medium sized tree possessing leaves which appear glabrous on both surfaces. The plant is rarely seen in wild-form and is usually cultivated to obtain its fruit which is edible. The fruit usually attains maximum of 1-inch length in wild-form in contrast to its cultivated form where it may reach up to the length of 3-inch long and is almost half as wide as its length (Chaudhry, 1969). This variety is distributed in punjab region of Pakistan. *Z. mauritiana* occurs in form of small shrubs to medium sized tree .The natural habitat of plant is warm subtropics and tropics of South Asia. where it exist in its wild form .The cultivated form of plant spreads through Indo-China and Southren China East ward whereas through Malesia it spreads to South East ward. In contrast Z. spina-christi belongs to drier tropical areas of Middle east, Ethiopia,North-East Africa and Eastren Africa. In Iran, Saudi Arabia and farther west Turkey it exist in its wild form. In India, Pakistan, Egypt, Syria, the Mahgreb, Saharan Oases and Zanzibar it exists as minor cultivated plant. (Azam-Ali *et al.,* 2006)

The species of *Ziziphus* are enriched in phytochemicals including various Vitamins like vitamin-C (Bakhshi and singh, 1974 : Singh *et al.,* 1973), vitamin-$B_1$ (Thiamine), vitamin-$B_2$ (Riboflavin) (Troyan and Kruglyakov, 1972; Kuliev and Guseinova, 1974), Alkaloids, (Pareek, 2001; Tschesche *et al.,* 1976; Tschesche *et al.,* 1979; Han *et al.,* 1990; Jossang *et al.,* 1996), Carbohydrates (Bakhshi and singh, 1974; Singh *et al.,* 1973) and heteropolysacchrides like Pectin-A (Tomoda *et al.,* 1985), Glycosides like Flavonoid Glycosides/ Spinosins (Woo *et al.,* 1979), various acids like Triterpenoic Acids (Lee *et al.,* 2003), Betulinic Acid (Pisha *et al.,* 1995; Kim *et al.,* 1998; Eizhamer and Xu, 2004), Oleanolic acid (Hsu *et al.,* 1997), Saponins like Glycoside saponin (Ogihara *et al.,* 1976), Phospholipids (Goncharova *et al.,* 1990), Inorganic minerals like Calcium and phosphorous (Bakhshi and singh, 1974; Singh *et al.,* 1973), metal ions like iron (Bakhshi and singh, 1974; Singh *et al.,* 1973), proteins and carotenes (Bakhshi and singh, 1974; Singh *et al.,* 1973).

Inflammation is a body response to various conditions like infection, injury or destruction which is accompanied by various outcomes like swelling, pain, heat, redness and disturbed physiological functions (Chandra *et al.,* 2012). It is considered as a normalresponse to tissue injury caused by microbial agents, physical traumaornoxious chemicals which is protective in nature. It is body's natural defense reaction to eliminate or limit spread of injurious agentsby inactivating or destroying invading organisms, by removing various irritants and by on setting stage for tissue repair. The release of chemical mediators from injured tissues and migrating cells will trigger inflammation process (Chandra *et al.,* 2012). There are two classes of inflammation acute and chronic. Enhanced vascular permeability, capillary infiltration and migration of leukocytes are all series of events occurring in process of acute inflammation. Whereas infiltration of mononuclear immune cells, neutrophils, macrophages and monocytes followed by fibroblast activation leading to proliferation and finally resulting in fibrosis are all among series of events occurring in chronic inflammation. Inflammation is among one of the common clinical condition whereas rheumatoid arthritis be better described as chronic deliberating autoimmune disorder (Nadkarni, 2000). Lifethreading hypersensitivity reactions and progressive organ damage are the potential harmful effects associated with inflammation (Robbins *et al.*, 2008). Non-steroidal anti-inflammatory drugs is the most common class of drugs used for the management of various inflammatory conditions NSAIDs have several side effects associated with them especially gastric irritation facilitating the formation of gastric ulcer later (Sankari *et al.,* 2009; Tripathi, 2008).

Medicinal plants are believed to be potential source of bioactive compounds which were used as pharmaceutical agent or as a lead compound in the process of drug development (Lulekal *et al.,* 2013). For the purpose of primary healthcare within developing countries 80% of populations rely on

herbal and traditional medicine according to World Health Organization (Zhang, 1998). Plants exert their beneficial effects due to presence of secondary metabolites which are considered as bioactive compounds exhibiting pharmacological potential (Said *et al.,* 2014). Need of hour is to obtain bioactive compounds by screening and exploring traditional medicinal plants which provide base for further pharmacological studies (Hemalatha *et al.,* 2013).

The traditional therapeutic use of plant medicine helps in the development of new and effective drugs by through scientific examination and validation of these plants which happen successfully in past (Koehan, 2009). A novel source of newer compounds with significant anti-inflammatory activities is presented by rich wealth of bioactive compounds in plant kingdom. Low cost, perceived efficacy and low incidence of serious side effects are among the major merits of herbal medicine (Chandra *et al.,* 2012).

*Ziziphus* is well known all across world for possessing anti-inflammatory potential since ancient time. Huangquin Tan containing fruit of *ziziphus jujube* is a compound prescription having marked anti-inflammatory effect (Huang *et al.,* 1990). *Ziziphus sina-christi* is widely used for managing pain and inflammation related problems all across gulf region as a popular traditional medicine (Asgarparah and Haghighat, 2012; Waggas and Al-Hasni, 2009; Azdu *et al.,* 2001). Ziziphus muritina is used as an anti-ulcer agent and alsopossess anti-iflammatory activity (Siddharth *et al.,* 2010; Ganachari and Shiv, 2004; Shiv *et al.,* 2004).

Bioactive metabolites vary in amount from plant to plant and in different parts of the same plant. Extraction of these metabolites depends upon two main factors i-e nature of extracting solvent and method adopted for extraction. In this study the extraction of bioactive metabolites was carried out by using five different solvents of varying polarity i-e methanol, acetone, ethylacetate, dichloromethane and chloroform. All the extracts were determined for TPC, TFC and anti-inflammatory potential by using different in-vitro assays like Inhibition of protein denaturation assay, Protease inhibition assay and Membrane stabilization by heat induced hemolysis and hypotonicity inducedhemolysis.

## Materials and method

*Collection of Plant Material*

*Ziziphus jujube* Gaertn (L) variety *hysudrica* Edgew was collected from Lahore, Pakistan. The bark of the root was separated from the inner root by physical means. Which was then shade dried for 10 days and ground, sieved and got properly stored in desiccator.

*Chemicals*

All the material and reagents used to conduct this study were taken from PCSIR Labs complex and University of the Punjab Lahore. All the chemicals were of AR grade and used as such without further purification.

*Preparation of plant extracts*

Hundred grams of each finely ground root bark and root of *Zizyphus Jujuba* (L.) Gaertn. Var. *hysudrica* Edgew was poured into six different flasks and extracted against various solvent having different polarities like: methanol, acetone, ethyl acetate, dichloromethane, *n*-hexane and chloroform. Flasks were allowed to continuously stir for 72 hours the material was then filtered and the resulting filtrates were air dried.

*Estimation of total phenolic content*

Folin-Ciocalteu reagent method was used to determine total phenolic content of all extracts. Single dilution of 200µg/mL for each dried extract was made in methanol. 200µL of dilution was then mixed with 400µL of Folin-Ciocalteu reagent in a volumetric flask. The solution was then mixed with 0.2mL of 7% $Na_2CO_3$ solution, after heating it at 25°C for 5-10 mins. The final dilution was done by using deionized distilled water and final volume was made upto 10ml in volumetric flask. The mixture was held at 25°C for 2 hours before taking absorbance at 765nm (McDonald *et al.,* 2001). Gallic acid was used as a standard for which calibration curve was plotted.

Equivalent of permg gallic acid (GAE) per gram of dried sample (mg/g) were calculated to determine the total phenolics.

*Estimation of total flavonoid content*

Initially 2 mL of methanol was used to dissolve 100µL of 200µg/mL of crude extract which was then diluted by using 4ml distilled water. Then 0.6ml of each 10% $AlCl_3$ and 5% $NaNO_2$ were added in above mixture which was then allowed to stay at ambient conditions for 10 mins. Final reaction mixture was made by adding 4mL of 1M NaOH and final volume of 20mL was made up by using distilled water. The absorbance was then measured at 510 nm after mixture was allowed to stand for 25 mins (Chang *et al.,* 2002). Rutin was used as a standard for which calibration curve was plotted. Equivalent ofmg Rutin per g of dried sample (mg/g) were calculated to determine total flavonoid.

*Estimation of Anti-inflammatory Potential*

Anti-inflammatory activity is performed by using various in-vitro methods which are described as follows

*Inhibition of protein denaturation*

1ml of each test solutions having single concentration (800µg/ml) were taken in test tube. Test solutions and standard drug was mixed with 1ml of egg albumin solution (1mM) and was subjected to incubation at 27± 1°C for 15 minutes. Denaturation will be induced by keeping the reaction mixture at 70°C in water bath for 10 minutes. Turbidity was measured spectrophotometrically at 660nm (Sakat *et al.,* 2010; Suchita *et al.,* 2017). The experiment was performed in triplicates. Percent inhibition of protein denaturation was calculated by using following formula

% inhibition protein denaturation = [(Abs control – Abs sample) /Abs control]* 100

*Anti-proteinase assay*

Reaction mixture (2ml) containing 0.06mg tripsin, 1ml 20mM Tris HCl buffer (pH 7.4) and 1ml test sample having single concentration (800µg/ml) was prepared. The mixture was incubated for 5min at 37°C after which 1ml 0.8% (w/v) casein will be added, then mixture was incubated additionally for 20mins. The reaction will be terminated by adding 2ml of 70% perchloric acid. Cloudy suspension will be centrifuged and the absorbance of supernatant would be read at 210nm against buffer as blank Sakat *et al.*, 2010; Mahabal and Kaliwal, 2017. The experiments will be performed in triplicate finally the percentage inhibition was calculated by using following formula.

% Inhibition = [(Abs control –Abs sample) /Abs control]* 100

*Membrane stabilization assay*

*Preparation of red blood cells (RBC's) suspension*

Fresh whole human blood (10ml) will be taken in heparin containing centrifuged tubes. The tubes will be centrifuged at 3000 rpm for 10 min and should be washed three times with equal volume of normal saline Sakat *et al.,* 2010; Mahawal and Kaliwal, 2017.

*Heat induced hemolysis*

The reaction mixture (2ml) was prepared by using 1ml of test solution and 1ml of test drug and 1ml of 10% RBC's suspension. To the control test tube only saline was added instead of drug. Reaction mixture containing centrifuge tubes were incubated in a water bath at 56°C for 30 min. test tubes were then cooled under running tap water at the end of incubation. The reaction mixture was centrifuged at 2500 rpm for 5min and the absorbance of supernatant was taken at 560nm Sakat *et al.,* 2010; Mahawal and Kaliwal, 2017. The experiment was performed in triplicates. Percent membrane stabilization or percent inhibition of hemolysis was calculated by using following formula

Percentage inhibition = [(Abs control –Abs sample) /Abs control]* 100

*Hypotonicity induced hemolysis*

Reaction mixture was prepared by using 0.5 ml hyposaline (0.42% NaCl), 1ml 0.15M sodium phosphate buffer, and pH 7.4, 1ml each test sample at single concentration (800µg/ml) and 0.5ml erythrocyte suspension. Reaction mixtures were mixed and incubated for 30 minutes at 56°C. Tubes were cooled under running water and then

centrifuged at 3000 rpm for 5 minutes. The absorbance of supernatant was taken at 560nm Suchita *et al.,* 2017; Mahawal and Kalwal, 2017. The experiment was performed in triplicates. Percent membrane stabilization or percent inhibition of hemolysis was calculated by using following formula

Percentage inhibition = [(Abs control –Abs sample) /Abs control]* 100

**Results and discussion**

*TPC and TFC of plant extracts*

All organic extracts of stem of *Ziziphus jujube* Gaertn (L) var. *hysudrica* Edgew have shown significant value of TPC and TFC (Fig. 1 and Fig. 2). Methanol, ethylacetate and acetone extracts differ significantly from eachother in term of TPC ($p< 0.05$). Whereas for TFC acetone and ethyl acetate extracts differ significantly from methanol extract ($p < 0.05$), methanol, dichloromethane and chloroform extracts differ significantly from acetone and ethylacetate extracts ($p < 0.05$), acetone, ethylacetate and chloroform extracts differ significantly from dichloromethane extract ($p < 0.05$) and acetone, ethylacetate and dichloromethane extracts differ significantly from chloroform extract ($p < 0.05$).

It was clearly shown by graphs that acetone extract has shown highest TPC (35mg gallic acid equivalent / g of extract) and TFC (60.59mg rutin equivalent/g of extract). Methanol extract was second high in term of TPC followed by dichloromethane, chloroform and ethylacetate extracts. Ethylacetate extract was second high in term of TFC followed by dichloromethane, methanol and chloroform extracts. The high concentration of phenolic content in methanol and acetone extracts reflects polar nature of these compounds. Whereas high concentration of flavonoid content in acetone, ethylacetate and dichloromethane extracts explore polar to intermediate polar nature of these compounds. Not all phenolics and flavonoids are polar these compounds cover range from polar to intermediate polar to nonpolar compounds (tocopherol is a nonpolar phenolic) that's why high concentration of phenolics and flavonoids were observed with dichloromethane and ethyl acetate extracts. It may be happen due to presence of polar and intermediate polar phenolics and flavonoids in abundance in plant as secondary metabolites.

*Anti-inflammatory activity of plant extracts*

*Inhibition of albumin denaturation*

All organic extractsof stem of *Ziziphus jujube* Gaertn (L) var. *hysudrica* Edgew have shown considerable inhibition of albumin denaturation (Fig. 3.) It was shown by graph only dichloromethane extract differ significantly from all other extracts in term of anti-inflammatory activity ($p < 0.05$). Acetone and methanol extracts were high in term of anti-inflammatory potential by showing 80% and 79.51% inhibition of albumin denaturation.% inhibition for other extracts by albumin denaturation assay was Chloroform 79.32%, ethylacetate 78.93% and Dichloromethane 74.87% respectively.

*Anti-proteinase assay*

All organic extracts of stem of *Ziziphus jujube* Gaertn (L) var. *hysudrica* Edgew have shown anti-proteinase activity (Fig. 4.) It was shown by graph that extracts do not differ significantly from each other in term of anti-inflammatory activity ($p< 0.05$). Dichloromethane and acetone extracts were high in term of anti-inflammatory potential by showing 96.70% and 96.16% inhibition of protease activity.% inhibition of other extracts by anti-protease assay was Methanol 96.08%, chloroform 95.88% and ethylacetate 95.32%

*RBC´s membrane stabilization by hypotonicity induced hemolysis model*

All organic extracts of stem of *Ziziphus jujube* Gaertn (L) var. *hysudrica* Edgew have shown RBC´s membrane stabilization effect against hypotonicity induced hemolysis (Fig. 5.) It was shown by graph that all extracts differ significantlyfrom each other in term of anti-inflammatory activity ($p< 0.05$). Chloroformand dichloromethane extracts were high in term of anti-inflammatory activity by showing 72.04% and 71.49% membrane stabilization.% membrane stabilization of other extracts by hypotonicity induced hemolysis model was ethylacetate 64.20%, acetone 57.12% and methanol 42.48% respectively.

*RBC´s membrane stabilization by heat induced hemolysis model*

All organic extracts of Ziziphus *jujube* Gaertn (L) var. *hysudrica* Edgew. Have shown RBC´s membrane stabilization effect against heat induced hemolysis (Fig.6.). It was shown by graph that all extracts differ significantly from each other in term of anti-inflammatory activity ($p< 0.05$). Acetone and methanol extracts were high in term of anti-inflammatory potential by showing 90.92% and 89.57% RBC's membrane stabilization.% stabilization of other extracts by heat induced hemolysismodel was dichloromethane 87.99%, chloroform 85.28% and ethylacetate 80.32% respectively.

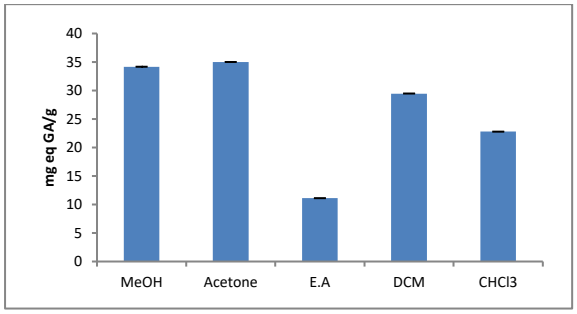


**Fig. 1.** Total phenolic content of stem of *Ziziphus jujube* Gaertn (L) var. *hysudrica* Edgew.

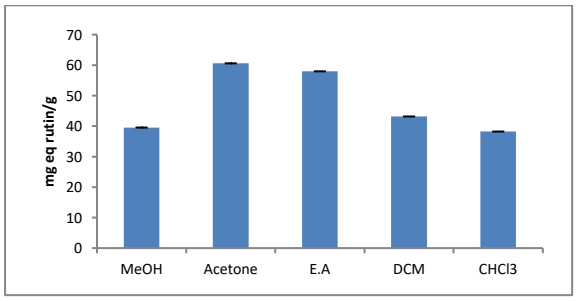


**Fig. 2.** Total flavonoid content of different extracts of stem of *Ziziphus jujube* Gaertn (L) var. *hysudrica* Edgew.

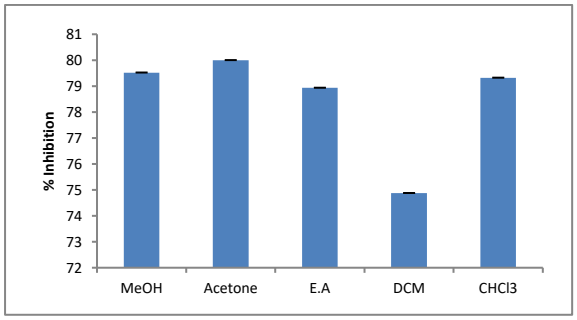


**Fig. 3.** Anti-inflammatory activity of different extracts of stem of *Ziziphus jujube* Gaertn (L) var. *hysudrica* Edgew by inhibition of protein denaturation assay.

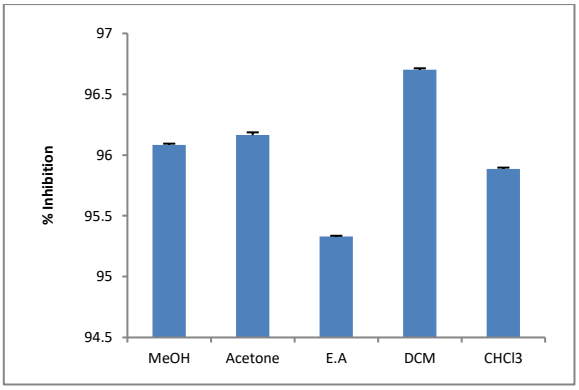


**Fig. 4.** Anti-inflammatory activity of different extracts of stem of *Ziziphus jujube* Gaertn (L) var. *hysudrica* Edgew by anti-proteinase assay.

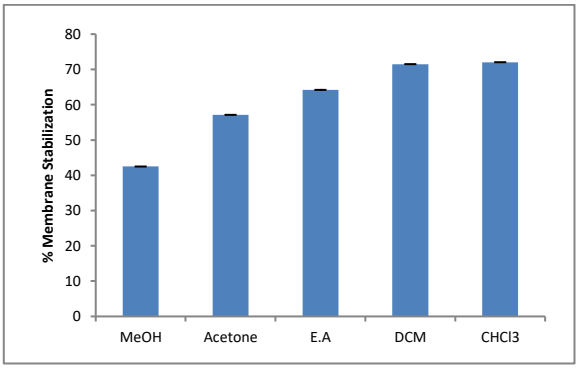


**Fig. 5.** Anti-inflammatory activity of different extracts of stem of *Ziziphus jujube* Gaertn (L) var. *hysudrica* Edgew by RBCs membrane stabilization (Hypotonicity induced haemolysis model).

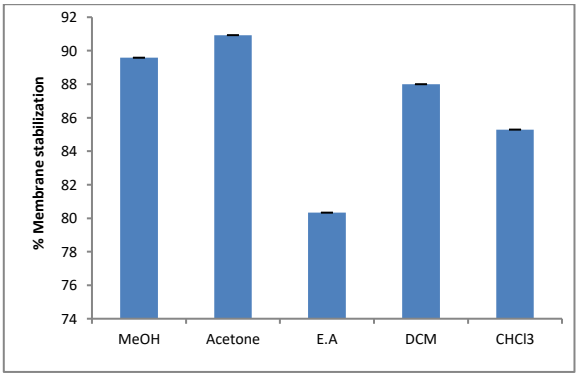


**Fig. 6.** Anti-inflammatory activity of different extracts of stem of *Ziziphus jujube* Gaertn (L) var. *hysudrica* Edgew by RBCs membrane stabilization (Heat induced haemolysis model).

Many infectious diseases like syphilis, leprosy, asthma, nephritis, tuberculosis, inflammatory bowel syndrome, celiac diseases, vasculitis and auto-immune diseases are commonly manifested by

inflammation (Das and Chatterjee, 1995). One of the well documented causes of inflammation is protein denaturation. A well-known fact about proteinase is that it embroils various joint responses. Off springs of proteinase are neutrophils, which contain numerous serine proteinases in there lysosomal granules. A critical part is assumed to be played by leukocyte proteinase aimed in fiery responses in the improvement of harm and proteinase inhibitors provide high level of insurance in this aspect (Vane and Booting, 1995). It was reported by researchers that one of the reason for rheumatoid joint pain is denaturation of protein, which is governed by production of auto-antigens in certain rheumatic sickness. It might also be a cause of denaturation of proteins in-vitro (Oyedepo and Femurawa, 1995; Grant *et al.,* 1970). Alteration of various atomicforces within protein molecules like electrostatic force, hydrogen bonding, hydrophobic bonds and disulfide bonds are all among possible mechanism of protein denaturation. Thermally induced protein denaturation was inhibited in a dose dependent manner by anti-inflammatory drugs (Chou, 1997; Grant *et al.,* 1970). Similar results were observed by many plant extracts (Sakat *et al.,* 2010). Inhibition of release of lyosomal content of neutrophils at the site of inflammation may be the possible mechanism of action through which plant extracts exert their anti-inflammatory effect. Lysosomal content of neutrophils is made up of bactericidal enzymes and proteinases,which may give rise to further tissue inflammation and damage upon extracellular release (Sarquis *et al.,* 2004; Chou, 1997).

Inflammation is accompanied by lysis of lysosomes membrane resulting in release of enzyme component causing variety of disorders. So stabilizing lysosomal membrane will help to reduce complicated inflammatory responses. An analogue to liposomal membrane is erythrocytic membrane (Chou, 1997). Lysis of red blood cells membrane resulting in hemolysis and oxidation of hemoglobin was achieved by exposing red blood cells to various deleterious factors like heat, hypotonic medium and various chemical substances like methyl salicylates or phenyl hydrazine (Lee *et al.,* 2010). Various plant extracts exert anti-inflammatory effect by inhibiting hypotonicity and heat induced red blood cells (RBC's) membrane lysis. Rupture of cell membrane due to excessive accumulation of fluid describes the hemolytic effect of hypotonic solution. Red cells got susceptible to secondary damage via free radical induced lipid peroxidation once there membrane got injured. Leakage of serum proteins and fluids into tissues is prevented by stabilizing membrane during a phase of enhanced permeability caused by various inflammatory mediators (Suchita *et al.,* 2017).

New chemical substances are believed to be present in medicinal plants having potential therapeutic effect. A fruitful and logical strategy for developing new anti-inflammatory drug having plant origin is to search the plants which were used traditionally for their anti-inflammatory effect (Godhandaraman and Ramalingam, 2016). *Ziziphus* is well known all across globe as a traditional medicine to treat various inflammatory conditions (Borgi *et al.*, 2007; Muhammad and Saeed, 2012). In present study stem of *Ziziphus* was selected to prepare various organic extracts by using solvents of different polarities. The extracts were then estimated for TPC & TFC and various in-vitro anti-inflammatory assays were performed to demonstrate anti-inflammatory potential of the plant. Results indicate that all extracts have shown anti-inflammatory potential to different extent depending upon nature of phenolics and flavonoids in them. In Albumin denaturation assay and RBC's membrane stabilization assay heat induced model the anti-inflammatory activity was associated with the presence of phenolics that's may be why acetone and methanol extracts have shown high activity by these models. Whereas anti-inflammatory activity by RBC's membrane stabilization hypotonocity induced model was associated mostly with flavonoids that's may be why ethylacetate and dichloromethane extracts have shown high activity by that model. Anti-inflammatory activity was also shown by both phenolics and flavonoids in synergism manner which best describe activity of chloroform extract by RBC's membrane stabilization hypotonicity induced model and dichloromethane extract by anti-protease assay.

The findings of this research are strongly supported by literature which clearly indicates phenolics and flavonoids as principal secondary metabolites present in plants which are responsible for anti-inflammatory activity (Oyine, 2018; Suchita *et al.*, 2017; Godhandaraman and Ramalingam, 2016; Djihane and Mihoub, 2016; Rehman *et al.*, 2015; Ayinke *et al.*, 2015; Vinchurkar *et al.*, 2014; Chawdhury *et al.*, 2014; Alhakmani *et al.*, 2014; Reshma *et al.*, 2014; Bhoomi *et al.*, 2013; Joseph *et al.*, 2013; Hossain *et al.*, 2012; Leelaprakash *et al.*, 2011; Mahesh *et al.*, 2011; Kumar *et al.*, 2011; Kumar *et al.*, 2011; Chippada *et al.*, 2011; Oyedapo *et al.*, 2010)

**Conclusion**

Extraction of stem of *Ziziphus jujube* Gaertn (L) var. *hysudrica* Edgew was done by using five different organic solvents of varying polarities (methanol, acetone, ethylacetate, dichloromethane and chloroform). Total phenolic content and total flavonoid content was estimated for all of the extracts. Acetone extract has shown high total phenolic and total flavonoid content. Anti-inflammatory potential of all extracts was measured by using three different in-vitro models (inhibition of albumin denaturation, anti-proteinase and RBC's membrane stabilization against heat induced and hypotonicity induced hemolysis assays). According to inhibition of albumin denaturation and RBC's membrane stabilization against heat induced hemolysis assays acetone and methanol extracts have shown high anti-inflammatory activity of 80% and 79.51% inhibition of albumin denaturation and 90.92% and 89.57% inhibition of heat induced RBC's hemolysis and the activity by these models was related to phenolics present in methanol and acetone extracts respectively. According to anti-proteinase assay dichloromethane and acetone extracts were high in term of anti-inflammatory activity by showing 96.70% and 96.16% inhibition of proteinase which was related to both phenolics and flavonoids present in dichloromethane and acetone extracts respectively. Whereas high anti-inflammatory activity 72.04% and 71.49% was associated with chloroform and dichloromethane extracts by using inhibition of hypotonicity induced RBC's hemolysis and was due to synergistic effect of phenolics and flavonoids.

**Declaration of conflict of interest**

None

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