doi.org/10.36721/PJPS.2020.33.2.SUP.887-894.1

Phytochemical, anti-inflammatory, anti-nociceptive and cytotoxic basis for the use of *Haloxylon stocksii*

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Abstract: A halophytic plant, *Haloxylon stocksii,* is used to treat various inflammatory disorders traditionally. The present study was carried out to investigate the phytochemical parameters, anti-inflammatory, analgesic and cytotoxic potential of the whole plant extracts of *H. stocksii*. The plant powder was standardized for pharmacognostic parameters. It was extracted with methanol followed by chloroform, ethyl acetate and water to prepare respective fractions. Total phenolic and flavonoid contents in the extract and fractions were estimated. The anti-inflammatory potential was determined through carrageenan-induced rat paw edema model. Centrally acting analgesic activity was assessed through the hot plate method. MTT assay was used to assess the viability of Human umbilical and human hepatocyte carcinoma cell lines upon exposure to plant extract/ fractions. Chloroform fraction showed the highest phenolic while ethyl acetate exhibited a maximum flavonoids content. The plant ethyl acetate fraction exhibited highest percentage inhibition of paw edema and maximum analgesic activity at 500 mg/kg dose. The plant methanolic extract and fractions showed dose dependent cytotoxic activity. The present study concludes that the extracts of *H. stocksii* may be effective and safe against acute inflammatory response and pain at therapeutic concentrations.

Keywords: Anti-inflammatory, analgesic, cytotoxicity, pharmacognostic evaluation, *H. stocksii.*

INTRODUCTION

Traditional medicines are used as an alternate therapy for the treatment of human and animal diseases throughout the developing countries (Saleem *et al*., 2020a). According to WHO, 80% population of developing countries depends on herbs for treatment of diseases. Moreover, 25% drugs listed in new pharmacopoeia are plant derivatives or semisynthetic compounds (Akhtar *et al*., 2019). Therapeutic actions of a huge number of medicinal plants have been identified and scientifically validated. Medicinal plants are therapeutically effective and ethnically accepted with minimal undesirable effects (Akhtar *et al*., 2017).

The plant *Haloxylon stocksii* (Boiss.) Benth. et Hook is a halophyte belongs to family Chenopodiaceae. It is found in the semiarid regions and widely distributed throughout South Asia and Middle East. It is often subjected to salinity, extremely variable drought and high temperature (Rathore *et al*., 2012). It is a perennial shrub, paniculately branched and used as animal forage (Baber et al., 2018). It is traditionally used to cure stomach aches, gastric troubles, ulcer and vomiting. The plant decoction is reported to be useful in arthritis, inflammation, resettlement of joints and tooth aches. It is also helpful to cure urinary tract obstruction especially in kidney and bladder stones (Hameed *et al*., 2011).

Plants produce abundant primary and secondary metabolites that include carbohydrates, organic acids, lipids, amino acids, terpenes, acyl lipids and phytosterols. All these plant metabolites play a pivotal role in essential functions of plant such as growth, respiration and photosynthesis and served as therapeutic moieties (Akhtar *et al*., 2019).

The constituents isolated and characterized from column chromatography of chloroform soluble fraction of *Haloxylon stocksii* are: Triacontanoic acid, β-sitosterol, ursolic acid, β-sitosterol 3-O-β-D-glucopyranosid, 1-Triacontanol, octadecanoic acid and dillenic Acid. Dillenic Acid is one of the important active constituents isolated from *H. stocksii.* Potent antifungal activity exhibited by the ethyl acetate soluble fraction of this species was due to the presence of steroidal glycosides. In

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brine shrimp lethality test, the methanolic extract of *H. stocksii* showed a significant cytotoxicity. Its chloroform-soluble fraction showed significant chymotrypsin inhibitory potential due to these isolated constituents, haloxylase. Strong chymotrypsin inhibitory activity was observed by further pharmacological screening of chloroform soluble fraction that led to the isolation and structural elucidation of two new sterols, Halosterol A and B6. Chloroform soluble fraction of *H. stocksii* (syn. *H. recurvum*), was non-competitive inhibitors of acetylcholinesterase and butyrylcholine enzymes due to newly isolated C-24 alkylated sterols given as haloxysterol A, B, C and D.

Inflammation is the healing process in response to tissue injury by irritants, pathogens and cell damage. It is the sequence of changes within a living tissue. An inflammatory response to tissue damage involves an intricate array of enzyme activation, release of mediators, cell migration, fluid extravasation, tissue breakdown and repair at host defense level (Saleem *et al*., 2019b).

Non steriodal anti-inflammatory drugs (NSAIDs) and opioids are used to treat inflammatory conditions and pain but due to occurrence severe adverse effects such as stomach pain, ulcer, kidney and liver damage etc. there is an immense need to find safe alternative therapy. A variety of medicinal plants are found effective as anti-inflammatory and analgesic agents over the last decade (Saleem *et al*., 2020b). The phytochemical, anti-inflammatory and analgesic evaluations of *H. stocksii* have not been documented previously. The objective of the current study was to evaluate phytochemical and pharmacological basis for use of *H. stocksii* to validate its ethnic use as anti-inflammatory and analgesic agent. Cytotoxicity potential was evaluated to confer its use as anticancer agent as well as *in vitro* toxicity.

MATERIALS AND METHODS

Collection and identification of plant

The whole fresh plant of *H. stocksii* was collected in March 2015 from Bahawalpur, Pakistan. The climate of Bahawalpur District is moderate during winter but very hot in summer season. Summer season usually extends from March to October and the maximum temperature ranges from 49°C to 52°C. Winters season starts from November and remains till February and is pleasant. The plant was identified from University of the Punjab, Lahore with Voucher no. LAH, 33-2015 and the specimen was deposited in herbarium for future reference.

Chemicals Used

Folin Ciocalteu's (FC) reagent (Unichem Chemicals, India), Gallic acid (Sinochem, China), Quercetin Methanol, Dimethyl sulfoxide (DMSO), Chloroform, Ethyl Acetate, Ferric chloride, chloroform, water, Sodium Hydroxide, Hydrochloric Acid, Nitric Acid, Sulphuric Acid, Distilled Water, Sodium Carbonate, Potassium Acetate and aluminum Chloride (Sigma Life Science, Germany) were used in the study.

Instruments

Herbal grinder (Bharat sales corporation, India), UV-vis spectrophotometer (Nanjing Safer Biotech, China), centrifuge machine (High speed KDC-140), rotary evaporator (RotoVap RE-200A), vortex mixture (Guangzhou, Four, China), glucometer (On Call® Extra, Acon Laboratories, USA), High performances liquid chromatography (HPLC) (Shimadzu, Japan), Fourier transform infrared spectrophotometer (FTIR Bruker, USA).

Extraction and fractionation

Extraction of *H. stocksii* ground powder (2kg) was carried out with methanol (1:5) through triple maceration. The extract was concentrated with a rotary evaporator at 45°C. The dried methanolic extract was dissolved in water and fractionated sequentially with chloroform and ethyl acetate. All the extracts were evaporated under reduced pressure and kept in a refrigerator until further use (Saleem *et al*., 2017a).

Phytochemical evaluation

Organoleptic evaluation of *H. stocksii* comprised of observing the texture, taste, appearance, color, shape, odor and fracture of the plant material. The physicochemical parameters such as moisture content, loss on drying and ash values were evaluated (Saleem *et al*., 2019a).

For moisture content, powdered plant material (2 g) was dried at 105°C in an oven for 5 h. The evaporating dish was cooled at room temperature until a constant weight was obtained. Dry matter and loss on drying were calculated. Total Ash value was calculated by heating plant powder (2 g) in tarred silica crucible at about 450°C for 15 min until carbon free ash was obtained. The acid insoluble and water-soluble ash were determined according to the previous method (Jegede *et al*., 2011).

Decoction of plant material was prepared. Impurities were removed by filtration and filtrate was used to assess foaming Index of *H. stocksii* according to the method described previously (Jegede *et al*., 2011). Foaming Index was calculated by the following formula:

$$\text{Foaming Index} = \frac{1000}{a}$$

Where,

α = the volume of decoction in ml used in a tube that produced foaming to a height of 1 cm.

Swelling Index showed the mucilage content in plant material and was determined according to the method described previously (Jegede *et al*., 2011).

Fluorescence analysis

Fluorescent properties of *H. stocksii* were evaluated by mixing plant powder (1g) in reagents such as 5% ferric chloride, chloroform, water, 1N NaOH, 1N HCl, 1N HNO_3, 1N H_2SO_4 and methanol (Gami and Parabia, 2010). The mixtures were incubated at room temperature in the UV chamber for 45 min and changes in color were observed under visible light, short (254 nm) and long (365nm) UV radiations.

Preliminary phytochemical screening

Extract and fractions of the plant were analyzed for the detection of alkaloids, tannins, terpenoids, phenolic compounds, lipids, proteins, steroids, carbohydrates, gums and mucilage following standard methods described previously (Saleem *et al*., 2017).

Total phenolic content (TPC)

Folin Ciocalteu's (FC) reagent (Unichem Chemicals) was used for determining TPC. FC (1ml) was added to methanolic solution of the plant extract (1mg/ml) and shaken vigorously. Sodium carbonate (7%, 10ml) was added to the mixture after 5 min and the solution was diluted up to 25 ml with distilled water (DW). Standard solutions (10, 20, 40, 80, 100, 120µg/ml) of Gallic acid (Sinochem, China) were prepared following the same procedure. DW used to prepare the blank solution. All the solutions were incubated at 25±2°C for 90min. The absorbance was measured at 750 nm wavelength. Mean value of absorbance was measured by preparing all the solutions in triplicate. Concentration of phenolics was estimated in µg/ml from the Gallic acid calibration curve and the phenolic content in the extracts was expressed as Gallic acid equivalent (mg of GA/g of extract) (Ahmed *et al*., 2017).

Total flavonoid content (TFC)

The standard solutions (10, 20, 40, 80, and 120 µg/ml) of quercetin (Sigma Life Science®, Germany) were prepared. Sample solutions (200 µl) were mixed with 1 M potassium acetate (100 µl), aluminium chloride (100 µl) and distilled water (4.6 ml). The treated solutions were incubated for 45 min at 25±02°C. The absorbance was determined at 415 nm post-incubation. The concentration of flavonoids was estimated from the Quercetin calibration curve. The flavonoid content present in the extract and fractions was expressed in terms of quercetin equivalent (mg of QE/g of extract) (Ahmed *et al*., 2017).

Animals

Anti-inflammatory and analgesic activities of the plant were carried out in Wistar rats (150-200 g) of both sexes. Animals were kept in polyvinyl cages at animal house of The University of Lahore at 25±2°C in 12 h light–dark cycles and had free access to standard food pellets and water. The study was approved by the Animal Ethics Committee of the University of Lahore and carried out according to the institutional guidelines. Animal study approval number was 2015-IREC-49 (b).

Anti-inflammatory activity

The effect of *H. stocksii* was assessed in Wistar rats using carrageenan as an edematogenic agent. Carrageenan suspension (1% w/v, 0.05 ml) was injected into the right hind paw of animals to induce edema. The animals were randomly divided into 10 groups with six animals in each group. Normal saline (NS) was administered to the negative control group, whereas diclofenac sodium (DS) (50 mg/kg) was given to the positive control group. Aqueous, methanolic, chloroform and ethyl acetate extracts of *H. stocksii* were given to test groups at 250 and 500 mg/kg dose. All the treatments were given orally 30 min before the administration of carrageenan suspension (Saleem *et al*., 2017). The paw volume was recorded instantly and 1, 2, 3 and 4h post-administration of carrageenan using plethysmometer. The % inhibition in edema was calculated (Saleem *et al*., 2017).

Analgesic activity

Different extracts of *H. stocksii* were assessed with the hot plate method for potential analgesic activity. Rats were divided into 10 groups with six animals in each group. NS was given to the negative control group, whereas tramadol (30mg/kg) served as the positive control. Methanolic, aqueous, chloroform and ethyl acetate extracts were evaluated for analgesic activity at 250 and 500 mg/kg dose.

Each rat was kept on the hot plate with the temperature maintained at 55°C ± 05°C. Withdrawal latency time was measured through observing the licking, jumping or the rotational movements of rat's hind paw at the baseline before the administering test extracts. Latency time was determined again at 15, 30, 60 and 120-min post-treatment. Withdrawal latency was recorded in seconds and any increase in time period was considered as indicative of analgesia. A cut off time of twenty seconds was used to avoid tissue injury (Saleem *et al*., 2017).

Cytotoxic activity

Human Cervical Carcinoma (HELA) and Human Hepatocyte Carcinoma (Hep G2) cell lines were used for *in vitro* cytotoxicity assay. MTT assay was used to assess the viability of HELA and HepG2 cells upon exposure to *H. stocksii* extracts. Cell lines were acquired from the Institute of Molecular Biology and Biotechnology, the University of Lahore. Normal control for cell viability was DMEM whereas 10% DMSO was negative control. The growth medium was removed. Plant extracts were dissolved in 5% DMSO. Extracts were diluted with twofold dilution method. All dilutions of *H. stocksii* extracts were prepared and tested in triplicate. Freshly prepared 20 µl MTT dye solution (0.25% w/v) was added to wells 24 h post incubation. Cells were incubated again

for 3 h. Cell culture medium was removed and 100 µl DMSO was added (Akhtar *et al*., 2017). Cell viability was determined from the absorbance at 570 nm with a micro plate reader. Cell survival percentage was calculated according to the method described previously (Saleem *et al*., 2016).

STATISTICAL ANALYSIS

Statistical analysis of the anti-inflammatory and analgesic activity was performed through one-way ANOVA followed by Tukey's Post Hoc evaluation using SPSS® version 17. The data were presented as mean and standard deviation.

RESULTS

Percentage yield

The percentage yield (%) of plant methanolic, chloroform, ethyl acetate and aqueous extract was 4.1, 0.68. 0.88 and 3.1% respectively. While consistency of all extracts was hard, dark brown color and had characteristic smell.

Table 1: Fluorescence analysis of *Haloxylon stocksii*

Sr. No	Reagents used	Visible range	Short wavelength	Long wavelength
1	D +HCL	Dark Brown	Dark Brown	Bluish Black
2	D + Distilled Water	Brown	Dark Brown	Dark Blue
3	D+ Methanol	Light Brown	NF	Dark Pink
4	D+ NaOH	Blackish Brown	NF	Aqua
5	D + Conc. HNO_3	Dark Orange	NF	Greyish Blue
6	D+ Conc. H_2SO_4	Black	NF	Blue
7	D + Chloroform	Light Brown	NF	Light Blue
8	D + Ferric Chloride	Bottle Green	Black	Blue

Where D: drug; NF: no fluorenscence

Table 2: Total Phenolic and total flavonoid contents in the *Haloxylon stocksii* extracts

Extract/Fraction	TPC (mg GA/g)	TFC ((mg QA /g))
Methanolic	262.58 ± 7.490	79.860±6.020
Chloroform	337.94 ± 6.831	115.27 ± 7.146
Ethyl acetate	286.37 ± 5.227	118.51 ± 5.466
Aqueous	67.42 ± 4.316	40.22 ± 3.243

Table 3: Anti-inflammatory effect of *Haloxylon stocksii* extracts

Treatment	Normal paw volume	Paw volume (ml) after administration of Plant extracts (percentage inhibition)				
		1 h	2 h	3 h	4 h	5 h
Negative control (Saline)	0.622 ± 0.022	1.134±0.043	1.522±0.035	1.72± 0.062	1.358±0.126	1.002±0.015
Standard (Diclofenac sodium 50 mg/kg)	0.522 ± 0.040	0.756±0.032**** (33.09%)	0.832±0.025*** (45.33%)	0.872±0.028**** (121.30%)	0.82±0.016*** (39.25%)	0.806±0.023*** (19.76%)
methanolic extract 250 mg/kg	0.658 ± 0.028	0.986±0.027** (12.74%)	1.146 ±0.046** (24.70%)	1.246±0.040** (99%)	1.154±0.046* (14.51%)	0.86 ±0.032** (14.37%)
methanolic extract 500 mg/kg	0.704 ± 0.097	0.92±0.019 *** (18.58%)	1.104±0.117* (27.46%)	1.22 ±0.019** (29.07%)	1.054±0.019*** (21.52%)	0.832±0.064** (16.96%)
Chloroform extract 250 mg/kg	0.584 ± 0.070	0.948±0.029** (16.10%)	1.126 ±0.036** (26.01%)	1.22 ±0.072** (29.21%)	1.13±0.052** (16.28%)	0.89 ±0.032** (11.37%)
Chloroform extract 500 mg/kg	0.720 ± 0.038	0.894±0.021*** (20.88%)	1.004±0.018*** (34.03%)	1.112±0.022*** (35.34%)	1.006±0.045*** (25.48%)	0.822±0.022** (17.96%)
Ethyl acetate extract 250 mg/kg	0.576 ± 0.062	0.932±0.013*** (17.52%)	1.11±0.037** (27.06%)	1.21 ±0.038** (29.65%)	1.12 ±0.042** (17.07%)	0.834±0.023** (16.56%)
Ethyl acetate extract 500 mg/kg	0.628 ± 0.049	0.792±0.024*** (29.91%)	0.87±0.027**** (42.83%)	0.942±0.047*** (45.23%)	0.888±0.049*** (34.24%)	0.800±0.029** (20.39%)
Aqueous extract 250 mg/kg	0.574 ± 0.058	0.98 ± 0.056** (13.27%)	1.16 ±0.111** (23.78%)	1.272±0.029** (26.04%)	1.1764±0.056** (12.87%)	0.908±0.015* (9.38%)
Aqueous extract 500 mg/kg	0.612 ± 0.075	0.942±0.022*** (16.63%)	1.164 ±0.108** (23.52%)	1.246±0.074** (27.55%)	1.12±0.014** (17.03%)	0.86±0.080** (14.17%)

Results expressed as mean ± standard deviation. Where *,**,***,**** $p<0.05$, 0.01, 0.001, 0.0001 respectively.

Table 4: Analgesic effect of *Haloxylon stocksii* shown by hot plate method

Treatment	Latency Time (Sec) After Administration of Plant extracts				
	0 min	30 min	60 min	90 min	120 min
Negative control (Saline)	6.092±0.046	6.412±0.075	6.672±0.051	6.926±0.035	5.922±0.051
Standard (Tramadol 30 mg/kg)	9.468±0.083**	10.14±0.047***	10.698±0.013***	11.32±0.019***	9.23±0.016***
methanolic extract 250 mg/kg	7.092±0.060*	7.714±0.018*	8.286±0.035**	8.90±0.029**	6.988±0.015*
methanolic extract 500 mg/kg	7.71±0.025*	8.79±0.024**	9.79±0.132**	10.236±0.046***	7.492±0.036**
Chloroform extract 250 mg/kg	7.382±0.018*	7.846±0.039*	8.408±0.013**	9.198±0.028**	6.794±0.027*
Chloroform extract 500 mg/kg	8.492±0.024**	9.219±0.019**	9.84±0.016**	10.43±0.032***	7.458±0.019**
Ethyl acetate extract 250 mg/kg	7.418±0.008*	9.918±0.015**	11.518±0.053**	12.308±0.172***	12.888±0.015**
Ethyl acetate extract 500 mg/kg	9.902±0.016**	11.946±0.018**	12.898±0.019**	14.51±0.027***	17.536±0.043****
Aqueous extract 250 mg/kg	7.026±0.063*	7.698±0.016*	8.25±0.034**	8.816±0.015**	6.912±0.019*
Aqueous extract 500 mg/kg	7.658±0.026*	8.75±0.035**	9.578±0.026**	10.12±0.049***	7.406±0.027**

Results were expressed as mean ± standard deviation. Where *,**,*** ,**** $p<0.05$, 0.01, 0.001, 0.0001 respectively.

Table 5: Cytotoxic potential of *Haloxylon stocksii* extracts and fractions

Extract/ fraction	Concentration (µg/ml)	Percentage cell viability of HepG2 (%)	Percentage cell viability of HELA cells (%)
Methanolic	25	55.40±2.70	98.70±3.44
	50	47.79±1.83	94.37±3.86
	100	46.80±3.33	66.72±6.12
Chloroform	25	83.13±3.28	89.26±7.12
	50	64.95±4.82	67.96±2.49
	100	59.98±2.56	60.67±5.82
Ethyl Acetate	25	98.47±4.32	99.03±1.79
	50	92.71±3.01	97.68±1.41
	100	86.44±1.34	90.67±5.13
Aqueous	25	81.03±5.20	98.19±2.03
	50	67.52±5.89	73.67±3.49
	100	64.50±3.50	67.62±2.03

Results were expressed as mean ± standard deviation. The % cell viability by all extracts was significantly ($P<0.05$) different from negative control (DMSO 5%) at all tested concentrations.

Pharmacognostic evaluation

Organoleptic evaluation of *H. stocksii* showed that the color of its fresh and dry stem was green and brown respectively. It was a cylindrical shrub of 5-15 cm length with an indistinctive odor and salty taste.

Physico-chemical analysis

Physico-chemical characteristics such as total ash (11%), acid insoluble ash (0.5%), water soluble ash (9.5%), swelling index (4.5%), moisture content (9%) and foaming index (102%) were calculated in the plant powder. All these characteristics are within official limits of WHO.

Fluorescence analysis

The plant was evaluated under short and long wavelength with different reagents to demonstrate the fluorescent characteristics. Results of fluorescent analysis are shown in table 1.

Phytochemical Analysis

Phytochemical analysis of *H. stocksii* confirmed the presence of alkaloids, phenolic compounds, carbohydrates and glycosides, amino acids, sterols and tannins in methanolic, chloroform, ethyl acetate and aqueous extracts.

TPC and TFC

TPC and TFC of the plant methanolic extract and fractions was calculated using regression equation $y= 0.0051x + 0.0475$, $R^2 = 0.9939$ and $y =0.0033x + 0.0054$, $R^2 =0.9912$ respectively. It was found that chloroform fraction exhibited the highest TPC while ethyl acetate fraction exhibited the highest TFC content in contrast to other extract/fractions as enlisted in table 2.

Anti-inflammatory activity

Anti-inflammatory potential of crude methanolic extract and fractions of *H. stocksii* was demonstrated in Wistar

rats through carrageenan induced paw edema method. Anti-inflammatory effect was studied at different time intervals and compared to control. The effect of ethyl acetate fraction was the most pronounced. All the extracts exhibited statistically significant ($p<0.05$-0.001) anti-edematous effect at the dose of 250 and 500 mg/kg compared to negative control (table 3). The maximum percentage inhibition of paw edema exhibited by diclofenac sodium and ethyl acetate extract at 500 mg/kg at 3 h in comparison to other extract. The plant extract reduced the paw edema in dose dependent manner. The order of anti-inflammatory activity was diclofenac sodium >ethyl acetate>chloroform>methanolic>aqueous.

Analgesic Activity

The extracts of *H. stocksii* and tramadol exhibited a dose dependent increase in latency time ($p<0.05$-0.001). Ethyl acetate fraction exerted significant anti-nociceptive activity at 250 and 500 mg/kg as compared to negative control. The latency time in rats treated with the extracts and tramadol increased significantly at all the specified time interval (table 4) in contrast to negative control.

Cytotoxic activity

The methanolic extract and fractions of *H. stocksii* were evaluated for their cytotoxic effect against HepG2 and HELA. The concentration and type of extract had significantly ($P<0.05$) affected the cell viability of HepG2. The concentration of extract also significantly ($P<0.05$) affected the cell viability of HELA. However, the type of extract did not significantly affect the cell viability of HELA. The effect of plant methanolic extract and fractions of *H. stocksii* on HepG2 and HELA cells viability was dose dependent and cell survival percentage was low at the highest concentration as shown in table 5.

DISCUSSION

Preliminary testing for the quality control of herbal drugs is elaborated in the form of various parameters such as extractive value, organoleptic evaluation, ash value, fluorescent analysis and phytochemical screening of the extracts. All these parameters play a key role to develop the validated monographs of medicinal plants (Gami and Parabia, 2010). This study demonstrated a low extractive value of *H. stocksii* that was attributed to the cold maceration. The moisture content of *H. stocksii* was within the official limit (8-14%) of WHO. The chances of bacterial and fungal contamination of the plant are minimal owing to low moisture content. It also allows long term storage of the plant material (Baber et al., 2018; Fatima *et al*., 2019). Adulteration of this drug with earthy material was assesed by acid insoluble ash. This study may serve as an appropriate source of information for identification of this medicinal plant (Ahmed *et al*., 2017).

The quantity of saponins in the plant material was confirmed by foaming index. The foaming index (102.04) of *H. stocksii* was also within the official limits of WHO (Gami and Parabia, 2010). Fluorescence under UV light serves as a useful tool for identification of powdered vegetable drugs. Pharmacognostic parameters of *H. stocksii* are similar to previous findings about the standardization of leaf and bark of *Holoptelea integrifolia* Roxb, flower of *Mesua ferrea* L, leaf and bark of *Anthocleista vogelii* (Jegede *et al*., 2011).

This study confirmed the presence of phenolic and flavonoid contents in *H. stocksii* which are directly related to antioxidant and anti-inflammatory activities of the plant through inhibition of free radicals (Saleem *et al*., 2019b). The presence of high saponin content in *H. stocksii* may be linked to possible antioxidant, immune-stimulant, hepato-protective, antibacterial, cytotoxic, antidiarrheal, anti-ulcerogenic and anti-oxytocic activities (Fatima *et al*., 2019). The absence of gums and mucilage in *H. stocksii* crude extract and fractions suggested inability of the plant to be used as natural excipient for traditional and novel dosage forms (Reddy and Manjunath, 2013).

The edema in the hind paw of rat after the carrageenan injection is a biphasic process in which several mediators (histamine, serotonin and bradykinin) work in sequence to produce inflammation. Histamine, serotonin and bradykinin are considered as primary mediators in the initial phase and prostaglandins are evident in the late phase of carrageenan-induced inflammation (Saleem *et al*., 2017a). Carrageenan-induced inflammation model in rats has long been used to investigate anti-inflammatory effect of agents such as NSAIDs that impede the production of prostaglandins. It can be suggested that the phenolic compounds in *H. stocksii* might had contributed to the anti-inflammatory effect (Saleem *et al*., 2019b). These compounds might have anti-inflammatory effect through inhibition of leukocyte response to immunologic reactions. Moreover, phenolic compounds scavenged oxygen derived free radicals by leukocytes (Saleem *et al*., 2020b). Oxygen derived free radicals play a key role in the inflammatory mechanism. These are involved in the activation of nuclear factor Kappa b which is responsible for the transcription of cyclooxygenase 2 and cytokines. The combined effect of free radical scavenging activities and pro-inflammatory enzyme inhibitors is responsible for the anti-inflammatory action of plant extracts (Saleem *et al*., 2020a). Hot plate method measured the complicated response to a non-inflammatory, acute nociceptive input and is selectively used for the evaluation of central nociceptive activity (Wadood *et al*., 2013). NSAIDs cannot be used as a standard therapy to investigate central analgesic activity as these acts as peripheral analgesic agents. The extension of hot plate latency is caused by centrally acting agents (Ibironke and Ajiboye, 2007).

Therefore, it can be speculated that the crude methanolic extract of *H. stocksii* and its fractions must have central analgesic activity. MTT assay is most widely used assay to access invitro cell viability, cytotoxic and anti-cancer potential of testing substance. The methanolic extract and fractions of *H. stocksii* were evaluated for their cytotoxic effect against HepG2 and HELA. Different concentrations and types of extracts had significantly ($P<0.05$) affected the cell viability of HepG2 and HeLa. All *H. stocksii* extracts showed dose-dependent cytotoxic effects, which proposed the therapeutic potential of this plant. The % cell viabilities of HepG2 and HELA was found maximum in ethyl acetate fraction (Kiran *et al.*, 2018). The existence of flavonoids, alkaloids, phenols and saponins might be attributed to the cytotoxic potential of *H. stocksii* (Akhtar *et al*., 2017). The plant extracts should be subjected to bioassay guided fractionations for isolating potential anticancer phytochemicals.

CONCLUSION

The detailed pharmacogenetic and phytochemical investigation of *H. stocksii* may be helpful in validation and quality control of plant raw material. It is concluded that ethyl acetate fraction of *H. stocksii* showed remarkable anti-inflammatory and analgesic effects. In the light of above findings, it is also concluded that the whole plant extracts of *H. stocksii* can be safely used as natural anti-inflammatory and analgesic agents. However, more detailed studies are needed to identify the active principles of *H. stocksii* and to elucidate its exact mechanism of action. Moreover, there is a great need to evaluate anticancer potential using different *in vitro* and *in vivo* assays.

REFERENCES

Ahmed S, Saeed-Ul-Hassan S, Islam M, Qureshi F, Waheed I, Munawar I, Ishtiaq S, Rasool S, Akhtar MF and Chishti SA (2017). Antioxidant activity of pistachia khinjuk supported by phytochemical investigation. *Acta. Pol. Pharma.,* **74**(1): 173-178.

Akhtar M F, Sharif A, Saleem M, Saleem A, Akhtar B, Raza M and Peerzada S (2017). Genotoxic and cytotoxic potential of *Alternanthera bettzickiana*, an important ethno-medicinal plant. *Cell. Mol. Biol. (Noisy-le-Grand, France)*, **63**(8): 109-114.

Akhtar M F, Ashraf M, Javeed A, Anjum A A, Sharif A, Saleem M, Mustafa G, Ashraf M, Saleem A and Akhtar B, Khan AM and Altaf I (2016b). Toxicity appraisal of untreated dyeing industry wastewater based on chemical characterization and short term bioassays. *B. Environ. Contamin. Toxicol.,* **96**(4): 502-507.

Akhtar MF, Ashraf M, Javeed A, Anjum AA, Sharif A, Saleem M, Mustafa G, Ashraf M, Saleem A and Akhtar B (2018). Association of textile industry effluent with mutagenicity and its toxic health implications upon acute and sub-chronic exposure. *Environ. Monit. Assess.,* **190**(3): 179-188.

Akhtar MF, Saleem A and Saleem M (2019). A comprehensive review on ethnomedicinal, pharmacological and phytochemical basis of anticancer medicinal plants of Pakistan. *Curr. Cancer. Drug. Tar.,* **19**(2): 120-151.

Baber A, Ahamd S, Rehman T, Ul Sabaha N and Arshad MA (2018). A review on phytochemical analysis and ethnobotanical uses of *Haloxylon stocksii. RADS Journal of Pharmacy and Pharmaceutical Sciences,* **6**(2): 162-167.

Fatima S, Akhtar M F, Ashraf K M, Sharif A, Saleem A, Akhtar B, Peerzada S, Shabbir M, Ali S and Ashraf W (2019). Antioxidant and alpha amylase inhibitory activities of *Fumaria officinalis* and its antidiabetic potential against alloxan induced diabetes. *Cell. Mol. Biol. (Noisy-le-Grand, France),* **65**(2): 50-57.

Gami B and Parabia M (2010). Pharmacognostic evaluation of bark and seeds of *Mimusops elengi* L. *Int. J. Pharm. Pharm. Sci.,* **2**(4): 110-113.

Hameed M, Ashraf M, Al-Quriany F, Nawaz T, Ahmad MSA, Younis A and Naz N (2011). Medicinal flora of the cholistan desert: A review. *Pak. J. Bot.,* **43**(2): 39-50.

Ibironke G and Ajiboye K (2007). Studies on the anti-inflammatory and analgesic properties of *Chenopodium ambrosioides* leaf extract in rats. *Int. J. Pharmacol.,* **3**(2): 111-115.

Jegede I, Ibrahim J & Kunle O (2011). Phytochemical and pharmacognostic studies of the leaf and stem-bark of *Anthocleista vogelii* (Planch). *J. Med. Plants Res.,* **5**(26): 6136-6139.

Kiran K, Saleem F, Awan S, Ahmad S, Ahmad S, Malik A, Ammar M, Akhtar B, Raza M and Peerzada S (2018). Anti-Inflammatory and anticancer activity of *Pteris cretica* whole plant extracts. *Pak. Vet. J.,* **38**(3): 225-230.

Rathore V, Singh J and Roy M (2012). *Haloxylon stocksii* (Boiss.) Benth. et Hook. f., a promising halophyte: Distribution, cultivation and utilization. *Gen. Res. Crop. Evolut.,* **59**(3): 1213-1221.

Reddy MR and Manjunath K (2013). Pharmaceutical applications of natural gums, mucilages and pectins: A review. *Int. J. Pharma. Chem. Sci.,* **2**(3): 67-71.

Saleem A, Akhtar M, Mushtaq M, Saleem M, Muhammad S, Akhtar B, Sharif A and Peerzada S (2016). Current trends in the treatment of hepatitis C: interventions to avoid adverse effects and increase effectiveness of anti-HCV drugs. *EXCLI J.,* **15**(1): 578-588.

Saleem A, Javeed A, Ashraf M, Akhtar M F, Akhtar B, Sharif A and Akhtar K (2017a). Anti-inflammatory, anti-nociceptive and antipyretic potential of *Terminalia citrina* fruit extracts. *Afri. J. Trad. Complemen. Altern. Med.,* **14**(5): 24-30.

Saleem A, Saleem M, Akhtar M F, Shahzad M and Jahan S (2020a). *Moringa rivae* leaf extracts attenuate Complete Freund's adjuvant-induced arthritis in Wistar rats via modulation of inflammatory and oxidative stress biomarkers. *Inflammopharma.*, **28**(1): 139-151.

Saleem A, Saleem M and Akhtar MF (2020b) Antioxidant, anti-inflammatory and antiarthritic potential of *Moringa oleifera* Lam: An ethnomedicinal plant of Moringaceae family. *South. Afr. J. Bot*., **128**(1): 246-256.

Saleem M, Ali H A, Akhtar M F, Saleem U, Saleem A and Irshad I (2019a). Chemical characterisation and hepatoprotective potential of *Cosmos sulphureus Cav. and Cosmos bipinnatus* Cav. *Nat. Prod. Res.*, **33**(6): 897-900.

Saleem A, Saleem M, Akhtar M F, Sharif A, Javaid Z, and Sohail K (2019b). *In vitro* and *in vivo* anti-arthritic evaluation of *Polystichum braunii* to validate its folkloric claim. *Pak. J. Pharm. Sci.,* **32**(3): 1167-1173.