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**ANTIMICROBIAL, ANTI-INFLAMMATORY AND ANTIPYRETIC ACTIVITY OF
CHORISIA SPECIOSA LEAVES. (BOMBACACEAE)**

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ABSTRACT

The aim of the study was to screen various solvent extracts of leaves of *Chorisia speciosa* to display its pharmacological activities in order to find possible sources for future novel compounds. Antibacterial and antifungal activity of *C.speciosa* was evaluated via disk diffusion method. Antipyretic and anti-inflammatory activities of plant were determined by using wistar albino rats. Rats were tested with doses of 200 and 400 mg/kg body weight (p.o.). Methanol and chloroform extract were found to be more effective than hexane extract. Significant antibacterial activity was shown against *B.cereus*, *S.aureus*, *K.pneumoniae* and *P.aeruginosa*, while methanol extract of *C.speciosa* showed noticeable zone of inhibition (11mm) against fungal *C.albicans* strain. In anti-inflammatory activity, inflammation was significantly ($p < 0.05$) antagonized by extracts of *C.speciosa*, at 400mg/kg. In antipyretic activity, chloroform extract of *C.speciosa* demonstrated dose dependently protection which is comparable to standard drug. Data from the present result revealed that *C.speciosa* has marked pharmacological activities.

Keywords: Antibacterial; Antifungal; Activity; Extract; Disk diffusion; Zone of inhibition; Anti-inflammatory; Antipyretic.

INTRODUCTION

C.speciosa is a species of deciduous tree belongs to family Bombacaceae. Phytochemical investigations of different parts of Bombacaceae species resulted in isolation of a variety of chemical

constituents produced by these plants. Anthocyanins, oxidized naphthalenes, flavonoids, alkaloids, amino acids, coumarins, sesquiterpenes, sesquiterpene lactones, long chain fatty

acids, triterpenes, steroids, lignans, cyclopropenoid fatty acids and carbohydrates are the most significant isolated phytochemical compounds [1]. They occur naturally throughout the tropical and subtropical regions of the world especially in the tropical America. Species of Bombacaceae family possess prominent medicinal, economic, and commercial values worldwide [2].

Over the centuries, phytochemical constituents have been used by different communities of the world [3]. Various local communities of different states of Pakistan have been using medicinal plants as a first source of their health care system and in fact these higher medicinal plants are used to treat a large number of ailments [4]. In Pakistan, this knowledge is well known in the name of Hikmat/Tabib while approximately, 600–1000 medicinal plants of the country have been used in the cure of different pathological health conditions by more than 40,000 registered and a considerable number of unregistered Hakims or Tabib[5]. This knowledge is based on experiences, without any proper scientific evidence and therefore, requires validation on scientific grounds [6].

Pathogenic bacteria have always been thought to be a considerable cause of morbidity and mortality in humans. Although different pharmaceutical

companies have introduced a number of new anti-bacterials in the last years, but resistance to these agents has also increased and has now become a worldwide problem [7]. Inflammation is a normal protective body response to tissue injury and compromise of a complex array of mediator release, cell migration, enzyme activation, tissue breakdown, fluid extravasations, and repair[8]. Harmful stimuli (pathogens, irritants or damaged cells) can initiate response of vascular tissue as inflammation. Inflammation is a protective effort by the organism in order to remove injurious stimuli as well as to initiate the healing process for the tissue [9]. Pyrexia or Fever is defined as rise in body temperature. It can be a response due to inflammation, malignancy or inflammation. Interleukin, cytokines, Tumor Necrosis Factor α (TNF- α) and interferon are formed in great amount under this condition, which increase PGE₂ which in turn triggers hypothalamus to increase body temperature [10]. Fever can be associated with the symptoms of sickness behaviour mainly sleepiness, inability to concentrate, lethargy, depression, and anorexia,. This increase in set point triggers increased muscle tone which in turn cause shivering. However antipyretic medication is effective for lowering the temperature which includes the affected person's comfort [11].

It is believed that current medication available such as non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are not effective in all cases of inflammatory disorders, because of their potency and side effects [12]. The aim of the present work is to report the in-vitro antimicrobial and in-vivo anti-inflammatory and anti-pyretic activity of the leaves of *C. speciosa* for the first time.

Methods

Chemicals

Culture media were purchased from Himedia, India. Dimethyl sulfoxide (DMSO) was purchased from Sigma Aldrich, USA. Methanol was obtained from Panreac, Spain. Chloroform and hexane were purchased from Sigma Aldrich, USA. Paracetamol (Tianjin Bofa Pharmaceutical Co, Lit., China), Diclofenac sodium (Suzhou Ausun Chemical Co, Lit., China), Acetic acid, Brewer's yeast (Merck Germany), Carrageenan (Sigma Lambda, USA) were used. Sterile normal saline was used in all in-vivo experiments as control while extracts were prepared in normal saline.

Micro-organisms tested

The bacterial strains of *Pseudomonas aeruginosa* (ATCC-27853), *Staphylococcus aureus* (ATCC-29213), *Escherichia coli* (ATCC-14028), *Bacillus cereus* (ATCC-11778), *Klebsiella pneumoniae* (ATCC-

10031), and *Salmonella enterica* (ATCC-14028) were used for the antibacterial assay whereas the fungal strain of *Candida albicans* (ATCC-14053) was used for carrying out the antifungal assay. All the microbial strains were supplied by Institute of Molecular Biology and Biotechnology, The University of Lahore.

Animals

Wistar albino rats of both sexes weighing from 150 g to 200 g were used for the in-vivo analysis of *C. speciosa*. They were obtained from the Animal house of The University Of Lahore. The animals were housed in polyvinyl cages with the strategy of having not more than six animals per cage and kept under the standard laboratory conditions maintaining temperature at $20 \pm 25^{\circ}\text{C}$ and a cycle of 12/12 h dark/light, received feed and water ad libitum. Analysis on animals was performed strictly in accordance with the guidelines which were provided by the Institutional Animal Ethics Committee.

Plant material

The leaves of *C. speciosa* were collected in the season of summer from Jinnah garden, Lahore, from Pakistan. The leaves were identified by Dr. Ajaib Choudhary, Professor in the Department of Botany, Government College University, Lahore. The voucher number that received after authentication of the plant was

GC.Bot.Herb. 2232. The leaves of *C.speciosa* were washed, cleaned and dried under shade and powdered in mechanical grinder. The powder was macerated with methanol, chloroform and n-hexane at room temperature. The extracts was then filtered by using a whatman filter paper (no.1), concentrated in vacuum under reduced pressure by using rotary flask evaporator. After the preparation of extracts, these were kept in the sterile bottles under refrigerated conditions, until its future use. The extracts were preserved at 2-4°C.

Antimicrobial assays

Antibacterial activity of methanol extract was determined by using disk diffusion method of Mbataet al, with some minor modifications [13]. Different stock solutions were prepared by dissolving 20 mg of the extract in sufficient amount of DMSO to make the final volume equal to 1 ml. 20 µl of the stock solution was impregnated to sterile paper disks (6 mm diameter) and then dried. Mueller-Hinton Agar (MHA) media was prepared and solidified in sterile Petri dishes. Different bacterial ATCC strain was swabbed onto the surface of the agar in each plate. The extract loaded disks were then placed on the surface of the swabbed agar media and the diameter of the zone of inhibition was measured after 24 h of incubation at 37°C. Antifungal

activity was evaluated by a method quite similar to the one used to determine the antibacterial activity [14]. Sabouraud Dextrose Agar (SDA) media was used instead of Mueller-Hinton Agar media and fungal ATCC strain was swabbed on the surface of the solidified agar media. Finally, the diameters of zone of inhibition were calculated after incubation at room temperature for 24h.

Acute toxicity

The acute toxicity test was carried out for *C.speciosa* to investigate any possible toxicity. The Wistar albino rats (n = 6) of either sex were administered with different doses (500, 1000 and 2000mg/kg, p.o.), while the negative control group received only saline (10ml/kg). All the groups were evaluated for any gross reaction for the first 4h and then mortality was noticed after 24h[15]. During the assessment time of 24 h, test rats were found normal and no gross reaction was seen.

Carrageenan-induced rat hind paw oedema

The anti-inflammatory activity was performed on Wistar albino rats of either sex (150–200g). The animals were randomly grouped in eight groups with the strategy that each group consisted on six animals [16]. Group I was treated with only normal saline (10ml/kg), group II was treated with Diclofenac sodium (10mg/kg),

remaining groups were treated with methanol (CSME), chloroform (CSCE) and hexane (CSHE) extracts of plant (200, and 400mg/kg, p.o). After thirty minutes of the above orally administration, carrageenan (1%, 0.05ml) was subcutaneously injected in the sub plantar tissue of the right hind paw of each rat. The inflammation of paw was measured by using digital vernier calliper instantly after the injection of carrageenan and then after 1,2,3,4 and 5h. The paw swelling in the drug treated animal as well as the animals treated with standard drug was compared with that of control and the percentage inhibition (anti-inflammatory activity) of oedema was evaluated using the formula:

$$\text{Percentage inhibition} = [1 - (a-x / b-y)] \times 100$$

a and x are the mean size of the right hind paw of the rats after and before carrageenan injection in the test group, b and y are the mean size of the right hind paw of the rats after and before carrageenan injection in the control group.

Anti-pyretic activity

The anti-pyretic property was evaluated for CSME, CSCE and CSHE by using Wistar albino rats (150-200g) of either sex. The selected animals for experiment were healthy and were acclimatized to standard laboratory conditions before the commencement of experiment. The animals

were classified into eight groups each had six rats. The normal rectal temperature of the body of each rat was recorded by using digital thermometer and then the pyrexia was induced in all rats by injecting 20% aqueous suspension of Brewer's yeast (10ml/kg s.c.). All the groups were fasted for all night but allowed each animal to free accesses to drinking water and after 24h the rectal temperature of each rat was recorded. The induction of fever was confirmed by the increase in temperature more than 0.5°C, and the animals that showed increase in temperature less than 0.5°C were removed from experiment [17]. Group I received only saline (10ml/kg) as a negative control, Group II received Paracetamol (150mg/kg) which was used as a standard drug while the rest groups received different extracts at the doses of 200 and 400mg/kg p.o. respectively. After the administration of drugs, rectal temperature was again recorded periodically at 1, 2, 3, 4 and 5h of the drugs administration. The percentage decrease in pyrexia was calculated by the following formula:

$$\text{Per cent reduction} = B - C_n / B - A \times 100$$

Where, B represents temperature after pyrexia induction; C_n temperature after 1, 2, 3, 4 and 5 h; where A is normal body temperature.

Statistical analysis

The results obtained were expressed as mean \pm SEM (Standard error of mean) of six animals. For statistical analysis, data was analysed by analysis of variance (ANOVA). Effects were considered to be significant at the $P < 0.05$ level.

RESULTS

Antimicrobial activity

Methanolic (CSME), chloroform (CSCE) and n-hexane (CSHE) extracts of *C. speciosa* leaves were tested against six bacterial strains (*B. cereus*, *P. Aeruginosa*, *K. pneumonia*, *E. coli*, *S. aureus*, and *S. enterica*) and the results are shown in table 1. Antibacterial activity was found to be highest for CSME and CSCE against *B. cereus*, with an inhibition zone of 16mm and 11mm, respectively. A moderate activity was shown against *P. aeruginosa*, *K. pneumonia* and *S. aureus* with an inhibition zone of ≥ 10 mm whereas no activity was found against *E. coli* and *S. enterica*.

Antifungal activity of the plant extract was also studied against *C. albicans* and results are shown in table 2. All three extracts CSME, CSCE and CSHE showed moderate antifungal activity with the inhibition zone 8mm, 10mm and 9mm, respectively.

Acute toxicity:

All three extracts: CSME, CSCE and CSHE were administered in mice with increasing dose manner and observed for any physical

or behavioural signs to evaluate acute toxicity potential of *C. Speciosa*. It was found safe at 500, 1000 and 2000mg/kg p.o. doses of extracts, comparable to the negative control group.

Anti-inflammatory activity

The anti-inflammatory activity of *C. Speciosa* was observed by administering different doses (200 and 400mg/kg p.o.) of CSME, CSCE and CSHE. All three extracts showed noticeable anti-inflammatory effect comparable to Diclofenac sodium (10mg/kg), as shown in table 3. Effect of CSCE was found more prominent at the dose of 400mg/kg showing significant reduction in carrageenan induced inflammation 2 hours after the administration of extracts was observed (figure 2).

Anti-pyretic activity

The antipyretic potential of *C. Speciosa* against yeast induced pyrexia was studied at different doses (200 and 400mg/kg p.o.) of CSME, CSCE and CSHE, as shown in table 4. A dose dependent effect was shown by all three extract with significant reduction after 3 h at 400mg/kg. Chloroform and methanol extract of *C. speciosa* leaves were found more effective as compared to hexane extract of plant with maximum percentage of inhibition comparable to Paracetamol (figure 4).

Table 1: Antibacterial activity of *C. Speciosa* on various ATCC strains

Bacterial strains	Zone of inhibition (mm)			
	CSME	CSCE	CSHE	Gentamycin
<i>B. cereus</i>	16	11	8	22
<i>P. aeruginosa</i>	9	9	-	20
<i>E. coli</i>	-	-	8	20
<i>K. pneumonia</i>	9	10	-	24
<i>S. aureus</i>	10	9	-	22
<i>S. enteric</i>	7	-	7	16

CSME: Methanol extract of *C.speciosa*; CSCE: Chloroform extract of *C.speciosa*; CSHE: Hexanes extract of *C.speciosa*; Disc diameter -6mm; Positive control: Gentamycin (10 µg /disc); --: no inhibition zone

Table 2: Antifungal activity of *C. speciosa* on ATCC strain

Bacterial strains	Zone of inhibition (mm)			
	CSME	CSCE	CSHE	Amphotericin B
<i>C. albicans</i>	8	10	9	21

CSME: Methanol extract of *C.speciosa*; CSCE: Chloroform extract of *C.speciosa*; CSHE: Hexanes extract of *C.speciosa*; Disc diameter -6mm; Positive control: Amphotericin B (10 µg disc)

Table 3: Effect of *C.speciosa* in Carrageenan induced inflammation in Rat's Paw

Treatment	Dose	Rat's Paw Size (mm)						
		Before Treatment		After Treatment				
		Normal	Inflamed	1 hr	2 hr	3 hr	4 hr	5 hr
Saline(negative control)	10 ml	4.58±0.12	6.00±0.11	6.02±0.21	6.11±0.11	5.98±0.22	5.97±0.11	5.74±0.20
Diclofenac Sodium	10mg	4.48±0.08	6.01±0.07	5.49*±0.17	5.12*±0.20	4.77**±0.14	4.67**±0.18	4.59**±0.17
CSME	200mg	4.51±0.02	6.00±0.05	5.98±0.23	5.75±0.02	5.63±0.12	5.61±0.03	5.51*±0.13
	400mg	4.61±0.04	6.03±0.02	5.77±0.13	5.40*±0.15	5.31**±0.15	5.30**±0.04	5.25**±0.01
CSCE	200mg	4.61±0.21	6.03±0.08	5.80±0.07	5.72±0.14	5.52*±0.07	5.45*±0.21	5.40*±0.11
	400mg	4.59±0.12	5.92±0.01	5.79±0.09	5.23**±0.11	5.07**±0.05	5.03**±0.11	4.98**±0.10
CSHE	200mg	4.59±0.10	5.94±0.10	5.91±0.17	5.85±0.08	5.62±0.07	5.61±0.11	5.41*±0.03
	400mg	4.48±0.09	5.90±0.20	5.76±0.15	5.31*±0.12	5.27*±0.13	5.21**±0.08	5.11*±0.10

CSME: Methanol extract of *C.speciosa*; CSCE: Chloroform extract of *C.speciosa*; CSHE: Hexanes extract of *C.speciosa*. Values are reported as mean ± S.E.M. for group of six animals. The data was analysed by ANOVA. Asterisks indicated statistically significant values from control. *P<0.05, **P<0.01

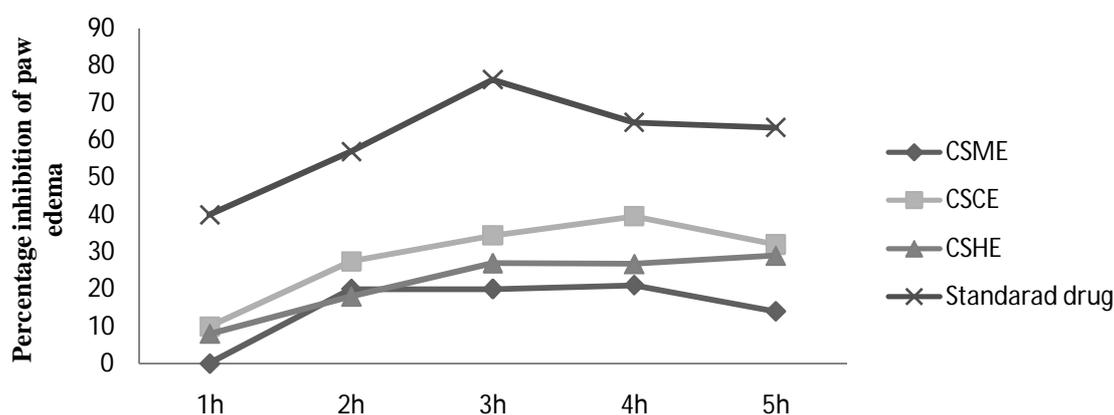


Figure 1: Anti-inflammatory effect of *C.speciosa* (200 mg/kg) in carrageenan induced paw oedema in rats. Each point shows per cent inhibition of paw oedema after 1,2,3,4 and 5h of treatment

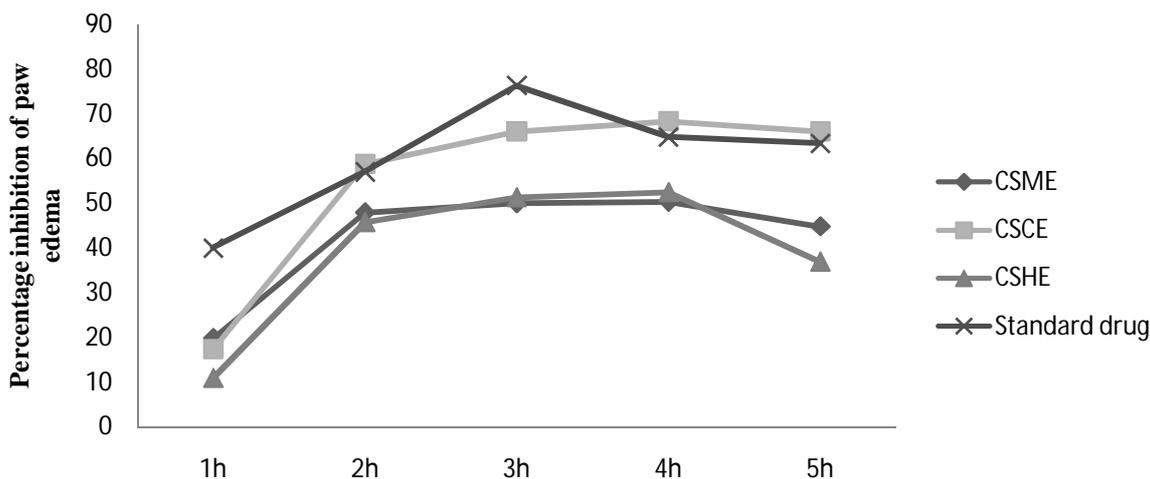


Figure 2: Anti-inflammatory effect of *C. speciosa* (400 mg/kg) in carrageenan induced paw oedema in rats. Each point shows per cent inhibition of paw oedema after 1,2,3,4 and 5h of treatment

Table 4. Effect of *C. Speciosa* on yeast induced pyrexia

Treatment	Dose	Rat's Rectal Temperature (°C)						
		Before Temperature		After Treatment				
		Normal	Pyrexia	1hr	2hr	3hr	4hr	5 hr
Saline	10 ml	37.00±0.12	38.92±0.11	38.80±0.21	38.77±0.11	38.65±0.22	38.65±0.11	38.70±0.20
Paracetamol	150mg	37.04±0.08	39.44±0.07	38.00**±0.17	37.58**±0.20	37.10**±0.14	37.19**±0.18	37.29**±0.17
CSME	200mg	37.06±0.02	39.70±0.05	39.44±0.23	39.40±0.02	38.98*±0.12	39.00±0.03	39.01±0.13
	400mg	37.00±0.04	39.00±0.02	38.61*±0.13	37.71*±0.15	37.65**±0.15	37.70**±0.04	37.61**±0.01
CSCE	200mg	37.02±0.21	39.84±0.08	39.54±0.07	39.20±0.14	38.98*±0.07	38.98*±0.21	39.00±0.11
	400mg	37.04±0.12	39.41±0.01	39.30±0.09	38.70*±0.11	37.71**±0.05	37.74**±0.11	37.80*±0.10
CSHE	200mg	37.00±0.10	39.30±0.10	39.22±0.17	39.01±0.08	38.87*±0.07	39.01±0.11	39.03±0.03
	400mg	37.08±0.09	39.40±0.20	39.00±0.15	38.96*±0.12	38.74*±0.13	38.61*±0.08	38.60*±0.10

CSME: Methanol extract of *C. speciosa*; CSCE: Chloroform extract of *C. speciosa*; CSHE: Hexanes extract of *C. speciosa*. Values are reported as mean ± S.E.M. for group of six animals. The data was analysed by ANOVA. Asterisks indicated statistically significant values from control. * $P < 0.05$, ** $P < 0.01$

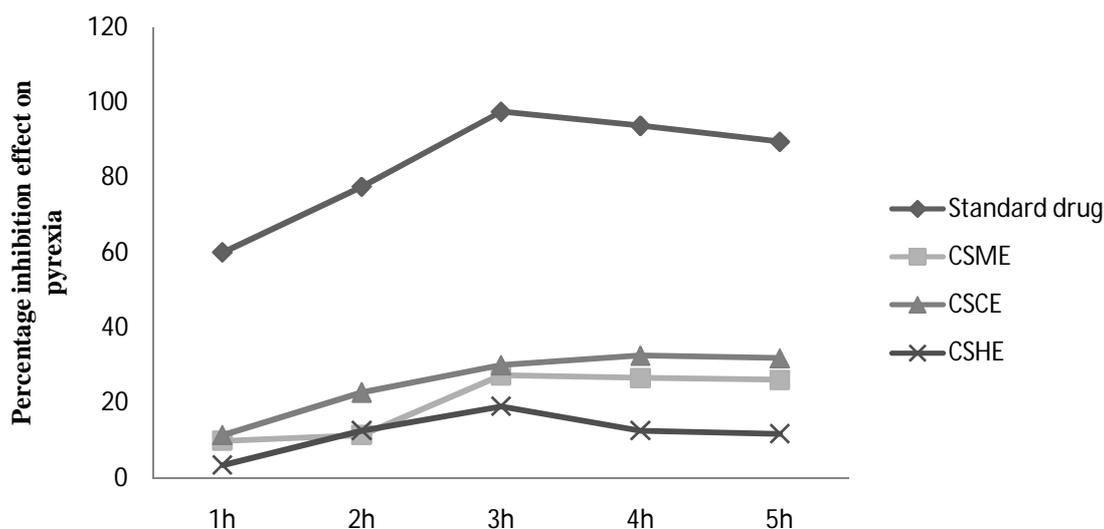


Figure 3: Anti-pyretic effect of *C. speciosa* (200 mg/kg) in yeast induced pyrexia after 1h, 2h, 3h, 4h and 5h in rats.

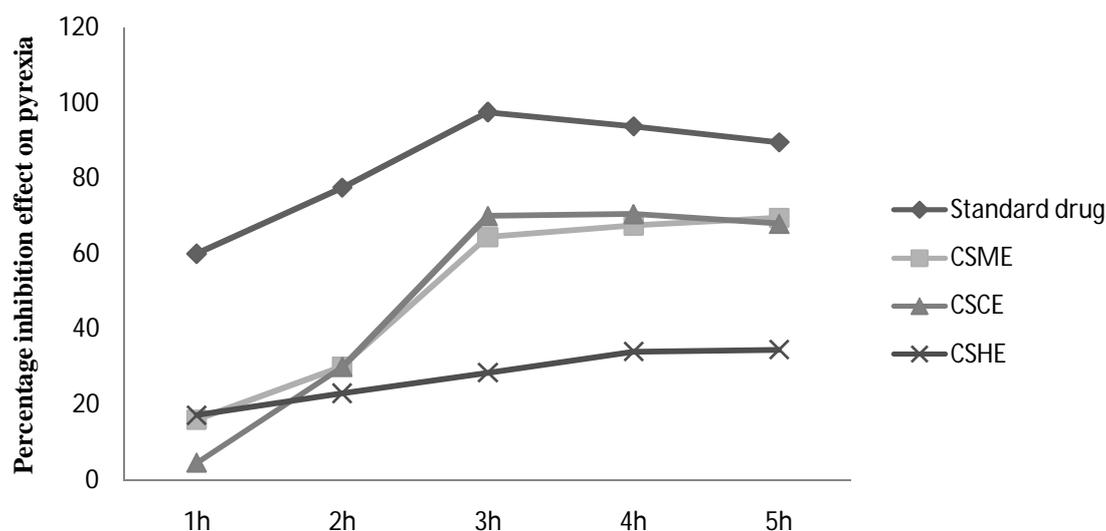


Figure 4. Anti-pyretic effect of *C. speciosa* (400 mg/kg) in yeast induced pyrexia after 1h, 2h, 3h, 4h and 5h in rats.

DISCUSSION

CSME and CSCE were found more effective against these bacteria as compared to CSHE

Results showed the presence of antibacterial activity in the plant thus indicating plant to be of medicinal value.

The results of recent analysis supports the traditional usage of the analysed plant and revealed that the plant extracts might have compounds that has prominent antimicrobial properties and can be used as antimicrobial agents in formation of new drugs for the therapy of infectious ailments caused by certain pathogens. Amongst the comparison of gram-positive and gram-negative bacteria, the gram-positive bacterial strains were more vulnerable to the extracts as compared to the gram negative bacteria [18]. The least activity of the extracts was shown against gram negative bacteria *P. aeruginosa* and no activity was shown against

S. enterica and *E. coli*. The antifungal ability was also determined by measuring the diameter of zone of inhibition recorded. Methanol extract of the plant were found to have maximum antifungal activity in comparison to hexane and chloroform extract.

Paw oedema induced by carrageenan is a well-known animal model to estimate the anti-inflammatory property of natural products as well as for the synthetic compounds. The oedema formation because of carrageenan in paw is significantly biphasic event that occur during 1–5h consist of two phases; the initial phase from 1h to 1.5h is prominently a non-phagocytic oedema that is followed by a second phase from 2h to 5 h with gradual increased oedema formation that can remained up to 5h [19]. The aetiology of initial phase is that oedema has been induced because of the action of mediators such as serotonin, histamine and bradykinin

on vascular permeability [20]. In the late phase or in the second phase oedema has been proving to be the consequence of over synthesis of prostaglandins [21]. The result of pre-treatment with *C. speciosa* showed that the extract (200 and 400mg/kg p.o.) is effective during the early phase of inflammation which happens because of release of histamine and serotonin. The anti-inflammatory ability of the extract remains noticeable up to 5th h of the analysis. Extracts revealed certain activity against carrageenan produce oedema in both of the phases.

Results of the present study revealed that the *C. speciosa* has considerable antipyretic and anti-inflammatory property with acceptable profile of safety. Injection of Brewer's yeast subcutaneously produces pyrexia by increasing the synthesis of prostaglandin. It is proved to be a useful screening test for the of plants materials as well as for the synthetic drugs to determine their antipyretic effect [19][22]. Pyrexia induced by yeast can also called as pathogenic fever and the reason of this could be the production of prostaglandins [23]. The administration of CSME, CSCE and CSHE via oral route moderately attenuated rectal temperature of rats induced by yeast. So that it can be postulated that extracts of *C. speciosa* consist of pharmacologically active

principles that interfere with the synthesis of prostaglandins. In the preliminary phytochemical analysis of the crude extract, important medicinal compounds like saponins, alkaloids, flavonoids, tannins etc. were found. Thus, the present findings can be associated to these groups of chemical constituents. Additional study is needed on *C. speciosa* to explore the accurate mechanism of action for its antipyretic and anti-inflammatory effects.

CONCLUSION

In conclusion, the extract *Chorisia speciosa* showed natural safe remedy treatment for pathological conditions. Current findings demonstrated scientific rationale for the use of the plant as antimicrobial, antipyretic and anti-inflammatory agent. Nevertheless, the further identification and isolation of pure secondary metabolites obtained from the plant will provide a source for understanding the mechanism of these activities and identification of lead compounds of therapeutic use.

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