

Antinociceptive, Antipyretic and Antimicrobial Activities of *Chrozophora tinctoria* Extracts

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Sher et al.: Pharmaceutical Evaluation of *Chrozophora tinctoria*

The antinociceptive, antipyretic and antimicrobial activities possessed by different solvent extracts of *Chrozophora tinctoria* were investigated. Analgesic activity of the crude methanol extract was studied using the hot plate and acetic acid-induced writhing methods and antipyretic activity using standard procedures on mice. *Chrozophora tinctoria* extract had highest latency time on the hot plate. The plant extracts reduced the number of abdominal constriction by 50 % at a dose of 150 mg/kg. The antipyretic effect of all doses tested was comparable to that of normal saline. Maximum decrease in the body temperature of the mice was observed at a dose of 300 mg/kg extract. Ethyl acetate extract revealed good antibacterial potential against *Salmonella typhi* (83 % inhibition) and *Shigella sonnei* (63 % inhibition). Chloroform extract showed highest antifungal activity against *Rhizopus* sp. (68 % inhibition).

Key words: Antibacterial, antifungal, antipyretic, *Chrozophora tinctoria*

People around the world appear to prefer herbal medicine over allopathy due to higher incidence of side effects. Herbal medicines could be regarded as safe to both the human health and the environment besides having both the aromatic and flavouring qualities. Herbs are being utilized for different remedial activities since ancient times. Initially medicinal plants were used in pure natural form (as teas, cataplasm, pulverized and other plant products)^[1,2]. The use of specific herbs for the treatment of different health issues were passed down from generation to generation in history. Medicinal herbs used for the contents of cocaine, codeine, digitoxin, and quinine, are still in use for various health needs^[3,4]. Medicinal plants contained active constituents that can be used as antimicrobial agents^[5-8,9]. The isolation, separation and classification of biologically active compounds from medicinal herbs is a continuing research.

Chrozophora tinctoria belongs to the family Euphorbiaceae, sub-family Acalyphoideae, tribe chrozophoreae and sub-tribe chrozophorinae. *Chrozophora tinctoria*, an annual plant is also known as, dyer's croton, giradol or turnsole^[10]. This plant is commonly found in different countries of the world including Africa, tropical Asia including India, Pakistan and Europe. *C. tinctoria* contained flavonoids, alkaloids,

coumarins and phenylpropanoid glycosides^[11,12]. *C. tinctoria* can be used for the treatment of different health issues including wound healing in diabetics^[13].

MATERIALS AND MEHTODS

Plants of *C. tinctoria* were collected from the mountains of Mohmand Agency, Khyber Pakhtunkhwa (KPK) Pakistan. The plant specimen was identified in the Department of Botany, Islamia College, Peshawar, Pakistan. The research project was approved by the Institutional Ethics Committee. All guidelines and Principles of Laboratory Animal Care (NIH publication No. 85-23, revised in 1985) were followed during the course of the experiment.

Crude extract preparation:

The plant materials were cut after washing with water, shade-dried and ground in an electric grinder. One kilogram of the powdered plant material was soaked in 5 l of methanol at 25° in the dark for 7 w, shaken

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thrice a day and filtered through a Whatman filter paper No.1. The solid remains was mixed with 2.5 l of fresh methanol and the whole process was repeated three times. All the filtered methanol extracts were mixed and evaporated under vacuum pressure at 45°. The crude extract was divided into two parts, one was used as the crude extract and the second was fractionated with different solvents.

Analgesic activity:

Albino mice of either sex (18-22 g) were used in the present investigation. The analgesic activity of the plant extract was determined using the hot-plate method^[14]. Thirty mice were randomly allotted to 5 treatment groups comprises of 6 mice each. First group was given the standard drug morphine, second received 0.9 % saline (control) and the remaining 3 groups were treated with plant extract at concentrations of 50, 100 and 150 mg/kg. All drugs were given orally as a suspension and normal saline and the standard drug was administered intraperitoneally (IP). An interval of 30 min was given to ensure proper absorption of the administered substances. The animals, which showed fore paw licking or jumping response within 6-8 s were selected for further investigation. The mice were put on the hot plate (55±0.5°) and the reaction time to thermal pain was noted. A cut off time (+10 s) was allowed to avoid thermal injury to the paws. The reaction time was recorded both before and 30, 60, 90, 120 and 180 min after the administration of the test or standard drugs.

The mean reaction time for each treated group was determined and percent increase in reaction time (% I) was derived using the Eqn., % I = ((It-Io)/Io)×100, where, It was the reaction time at time t, and Io was the reaction time at time zero (0 h). The mice were subjected to the same test procedure at +30, +60, +120, and +180 min after the administration of test/standard/control drug^[15]. Mice were sacrificed by cervical dislocation at the end of the experiment.

Mice of either sex weighing 18-22 g were used to measure analgesic activity using the acetic acid (AA)-induced writhing test. Mice were withdrawn from food and water 2 h before the start of the experiment. Mice were divided into 5 groups, each group consisting of 6 animals. First group served as the negative control (normal saline ml/kg), second group as positive control (aspirin 50 mg/kg), while third, fourth and fifth group received the plant extract orally in concentration of 50, 100, 150 mg/kg. Writhing behaviour was observed in mice after administering 1 % AA, IP. Number of

abdominal constrictions occurring over the period of 20 min was counted after 5 min of administration of 1 % AA (10 ml/kg)^[16]. The plant extract (50, 100, 150 mg/kg), aspirin or 0.9 % sodium chloride was administered IP 30 min before 1 % AA administration. Percent analgesia was calculated using the Eqn., % protection = 100-(number of writhes in experimental group×100/number of writhes in control).

Antipyretic activity of the crude extract of *C. tinctoria* was determined following the method of Adams *et al.*^[17]. Normal body temperature of each mouse was noted and pyrexia was induced in mice by injecting 20 % aqueous suspension of Brewer's yeast (10 ml/kg) subcutaneously. All groups were withdrawn from food overnight. However, accesses to drinking water was allowed and rectal temperature of each mouse was noted after 24 h. Rise in temperature more than 0.7° F was regarded as induction of pyrexia. For different treatments, the mice were divided into 5 groups, 6 mice each. Group I was treated with saline (10 ml/kg) as a negative control, group II received paracetamol (150 mg/kg) as the standard drug while the remaining groups (III, IV and V) were treated orally with plant extracts in 100, 200 and 300 mg/kg concentrations, respectively. Rectal temperature was again noted at 0.5, 1, 2, 3 and 4 h after treatment.

Antimicrobial activity:

Antibacterial activity of different fractions was determined by disc diffusion methods as reported in Khajuria *et al.*^[18] and antifungal activity by Ramdas *et al.*^[19]. Plates containing nutrient agar media were inoculated with 1 d cultures of microbial inoculums. Three Whatman No. 1 filter paper discs (6 mm diameter) were placed on the media in Petri plates. Test plant samples in 1 and 2 mg/disc concentration (6 and 12 µl volumes) were applied on to the discs. Standard drugs (ciprofloxacin and clotrimazole at dose of 50 µg per 12 µl) as the positive control and DMSO (12 µl/disc) as the negative control were also applied on the discs. Inoculated plates were kept at 37° for 18-24 h and zones of inhibition were recorded in millimetre in each plate.

Statistical analysis:

Data was expressed as mean of triplicate determinations. For statistical analysis, MSTATC computer software was used^[20]. Standard error of the means and level of significance (p<0.05) were calculated for each treatment.

RESULTS AND DISCUSSION

The crude methanol extract of *C. tinctoria* was screened for analgesic activity using the hot plate method in doses of 50, 100 and 150 mg/kg (Table 1). The data showed that the mean pain latency time of the 50 mg/kg extract-treated group was 13 ± 0.89 at 0 min. After administering the extract, the latency time was found to be increased to 14.83 ± 2.136 and 14 ± 2.097 s at 30 and 60 min compared to initial latency time of the same group or the normal saline group. The data also indicated that similar rise in latency was observed after 90 and 120 min (9.43 ± 1.47 s). However, latency time increased to 12.33 ± 2.065 s after 180 min. Similarly, the latency time of the plant extract in 100 mg/kg dose group was noted as 11.83 ± 1.47 s at 0 min. Rise in reaction time was observed after 30, 60 and 90 min measuring 12.66 ± 0.816 , 13.66 ± 1.21 , 16.33 ± 0.516 s compared with initial reaction time and latency time of saline group. The extract of 150 mg/kg showed significant ($p < 0.05$) effects after 60, 90 and 120 min showing increase in latency time of 15.66 ± 2.065 , 17 ± 1.26 and 15 ± 2.96 s compared to the initial time noted at 0 min and saline group. Morphine-treated groups significantly ($p < 0.05$) increased pain latency induced by the hot plate. Significant ($p < 0.05$) effects were also shown by 100 mg/kg plant extract after 60, 90 and 120 min compared to morphine-treated group. Similar results were also noted for 150 mg/kg concentration after 60, 90 and 120 min.

The data indicated that the mean number of writhes in control group was 75.83 ± 3.65 when tested AA-induced

method (Table 2). Aspirin at the dose of 100 mg/kg showed 74.08 % inhibition of abdominal constriction resulting in the mean number of 19.66 ± 2.160 writhings. The crude methanol extract at 50 mg/kg dose resulted in 18.47 % inhibition of abdominal constriction and showed lower mean number of writhes i.e. 61.83 ± 2.31 . Similarly, 100 mg/kg concentration inhibited the abdominal constriction up to 26.16 % resulting in the mean numbers of 56 ± 4.049 writhes. The crude extracts at 150 mg significantly ($p < 0.05$) reduced the number of abdominal constrictions to 38 ± 4.85 resulting in 49.89 % inhibition of writhes.

The antipyretic activity of *C. tinctoria* crude extract was evaluated at different concentrations in mice (Table 3). The mean temperature of control group prior to Brewer's yeast injection was $99.03 \pm 0.33^\circ$ F and the average temperature increased to $102.01 \pm 0.36^\circ$ F after Brewer's yeast injection. After 30 min, a gradual rise in the body temperature of the control group was noted ($102.28 \pm 0.21^\circ$ F). Similarly, a slight fall in body temperature was observed measuring $101.95 \pm 0.30^\circ$ F, $101.65 \pm 0.30^\circ$ F, $101.4 \pm 0.32^\circ$ F, and $101.03 \pm 0.186^\circ$ F after 1, 2, 3 and 4 h, respectively. The average temperature of paracetamol group before Brewer's yeast injection was $98.91 \pm 0.24^\circ$ F, which raised to $102.43 \pm 0.41^\circ$ F after brewer's yeast. After injection of paracetamol, a continued fall in the rectal temperature of mice was recorded in a time-dependant manner. The rectal average temperature of paracetamol group was $102.11 \pm 0.42^\circ$ F, $101.95 \pm 0.30^\circ$ F, $100.3 \pm 0.39^\circ$ F, $100.2 \pm 0.54^\circ$ F and $99.41 \pm 0.37^\circ$ F, at 0.5, 1, 2, 3 and 4 h, respectively.

TABLE 1: EFFECT OF CRUDE METHANOL EXTRACT OF *CHROZOPHORA TINCTORIA* ON THERMALLY INDUCED PAIN IN MICE

Group	Dose mg/kg	Mean pain latency (seconds)					
		0 min	After 30 min	After 60 min	After 90 min	After 120 min	After 180 min
Saline	----	10.83 ± 1.32	11.33 ± 0.81	11.66 ± 0.81	11.66 ± 1.63	10.33 ± 2.160	11.83 ± 1.169
Morphine	5	12.16 ± 1.72	16.5 ± 1.87	18.16 ± 1.60	19.33 ± 1.36	11.5 ± 1.37	11.83 ± 1.72
<i>Chrozophora tinctoria</i> extract	50	13 ± 0.89	14.83 ± 2.136	14 ± 2.097	9.83 ± 1.47	9.83 ± 1.47	12.33 ± 2.065
	100	11.83 ± 1.47	12.66 ± 0.816	13.66 ± 1.21	16.33 ± 0.516	13.33 ± 1.032	13.33 ± 1.21
	150	11.83 ± 1.47	13.16 ± 2.31	15.66 ± 2.065	17 ± 1.26	15 ± 2.96	12.66 ± 2.065

Crude methanol extract of *C. tinctoria* was tested at 50, 100 and 150 mg/kg doses. Values are expressed as mean \pm SEM

TABLE 2: EFFECT OF CRUDE METHANOL EXTRACT OF *CHROZOPHORA TINCTORIA* ON ACETIC ACID-INDUCED WRITHING IN MICE

Groups	Treatments	dose (mg/kg)	Number of writhes mean \pm SEM	Inhibition (%)
Control	Normal saline	----	75.83 ± 3.65	----
Standard	Aspirin	50 mg	19.66 ± 2.160	74.08
		----	61.83 ± 2.31	18.47
Crude methanol extract	----	100 mg	56 ± 4.049	26.16
		150 mg	38 ± 4.85	49.89

Crude methanol extract of *C. tinctoria* was tested at 50, 100 and 150 mg/kg doses. Values are expressed as mean \pm SEM

TABLE 3: ANTIPYRETIC ACTIVITY OF CRUDE METHANOL EXTRACT OF *CHROZOPHORA TINCTORIA*

Treatment	Dose mg/kg	Average temperature (°F) before Brewer's yeast injection	Average temperature (°F) after Brewer's yeast injection	Average temperature (°F) after Brewer's yeast injection at different time intervals				
				After 0.5 h	After 1 h	After 2 h	After 3 h	After 4 h
Control/saline		99.03±0.33	102.01±0.36	102.28±0.21	101.95±0.30	101.65±0.30	101.4±0.32	101.03±0.186
Paracetamol	5	98.91±0.24	102.43±0.41	102.11±0.42	100.78±0.63	100.3±0.39	100.2±0.54	99.41±0.37
Crude methanol extract	100	99.11±0.147	101.91±0.47	101.78±0.40	101.78±0.40	101.4±0.37	100.55±0.39	100.2±0.309
	200	98.61±0.37	101.98±0.49	101.35±0.472	100.78±0.44	100.51±0.594	100.05±0.50	100.05±0.686
	300	99±0.282	101.85±0.266	101.15±0.27	100.1±0.45	99.51±0.306	99.51±0.26	99.25±0.20

Crude methanol extract of *C. tinctoria* was tested at 50, 100 and 150 mg/kg doses. Values are expressed as mean±SEM

Similarly, the rectal temperature was also recorded in groups that received crude extract doses of 100, 200 and 300 mg at different time intervals. At 100 mg dose the average temperature before Brewer's yeast injection was 99.11±0.147° F, which increased to 101.91±0.47° F after the treatment. The rectal temperature of the mice was 101.78±0.40° F, 101.78±0.40° F, 101.4±0.37° F, 100.55±0.39° F and 100.2±0.309° F after 100 mg dose after 0.5, 1, 2, 3 and 4 h, respectively. However, it showed a decrease in temperature from 101.91±0.47° F to 100.55±0.39° F after 3 h. The average temperature before Brewer's yeast injection was 98.61±0.37° F, which raised to 101.98±0.49° F by administering 200 mg dose. Similarly, the rectal temperature was 101.35±0.472° F, 100.78±0.44° F, 100.51±0.594° F, 100.05±0.50° F and 100.05±0.686° F, respectively in 200 mg dose after 0.5, 1, 2, 3 and 4 h. The average temperature before Brewer's yeast injection was 99±0.282° F in 300 mg dose and after Brewer's yeast induction the temperature became 101.85±0.266° F. A decrease in temperature was noticed after 0.5, 1, 2 3 and 4 h (101.15±0.27° F, 100.1±0.45° F, 99.51±0.306° F, 99.51±0.26° F and 99.25±0.20° F, respectively). A significant ($p < 0.05$) antipyretic effect was noted in 300 mg dose. It showed 1.64° F fall in temperature after 2 h and came close to the normal body temperature of mice after 4 h.

Crude methanol extract, petroleum ether, chloroform, ethyl acetate, n-butanol and aqueous extracts of *C. tinctoria* were tested against *Escherichia coli* for possible antibacterial activity (fig. 1A). Almost all the extracts showed activity (zone of inhibition) against *E. coli* at concentrations of 1 and 2 mg/disc except the aqueous extract. Crude methanol extract showed 23.33±0.50 % zone of inhibition in concentration of 1 mg/disc and 33.33±0.70 % in 2 mg/disc. Chloroform extract produced 20.00±0.35 and 46.70±0.56 % inhibition zones at 1 and 2 mg/disc, respectively.

Similarly, the zone of inhibition for ethyl acetate extract was 30.00±0.62 % in 1 mg/disc and 40.00±0.56 % in 2 mg/disc. These results also revealed that most of the extracts showed activity against *Salmonella typhi* at both concentrations except with chloroform and aqueous extracts (fig. 1B). Crude methanol extracts showed 23.33±0.62 % zone of inhibition in 1 mg/disc and 56.70±0.30 in 2 mg/disc concentration. Ethyl acetate extract showed 63.33±0.30 % zone of inhibition in 1 mg/disc and 83.33±0.20 % zone of inhibition in 2 mg/disc compared to controls.

Crude methanol, n-butanol and ethyl acetate extracts revealed activity against *Pseudomonas aeruginosa* at the tested concentrations (fig. 1C). However, petroleum ether, chloroform and aqueous extracted samples did not show any activity at both concentrations. Crude methanolic extracts measured 23.33±0.20 % zone of inhibition in 1 mg/disc and 26.70±0.50 % in 2 mg/disc concentrations. Ethyl acetate extracted samples revealed 26.70±0.20 and 30.00±0.20 % zones of inhibition in 1 and 2 mg/disc, respectively. All extracts obtained from *C. tinctoria* showed activity against *Providencia* sp. at both concentrations (fig. 1D). Crude methanolic extract measured 43.33±0.40 % zone of inhibition in 1 mg/disc and 53.33±0.62 % in 2 mg/disc concentrations against *Providencia* sp. Petroleum ether extracts showed 36.70±0.50 and 43.33±0.50 % zones of inhibition at 1 and 2 mg/disc, respectively.

Crude methanol, petroleum ether, chloroform, ethyl acetate, n-butanol and aqueous extracts demonstrated different zones of inhibition against *Proteus mirabilis* in 1 and 2 mg/disc concentration except chloroform and aqueous extracts (fig. 1E). Crude methanol extract gave 20.00±0.50 and 23.33±0.80 % zones of inhibition in 1 and 2 mg/disc, respectively. Similarly, ethyl acetate extract showed 16.70±0.50 and 23.33±0.50 % zones of inhibition in 1 and 2 mg/disc. n-Butanol extract showed activity of 13.33±0.20 and 40.00±

0.40 % in 1 and 2 mg/disc concentrations against the same microbe. The data also revealed that crude methanol, petroleum ether and ethyl acetate extracts reduced the growth of *Shigella sonnei* but not chloroform, n-butanol and aqueous extracts (fig. 1F). Highest antibacterial activities were shown by crude methanol and ethyl acetate extracts compared to control. Crude methanol extract yielded 20 ± 0.20 and 40 ± 0.62 % zones of inhibition in 1 and 2 mg/disc concentrations, respectively. Similarly, ethyl acetate extract exhibited antibacterial activity of 53.33 ± 0.10

and 63.33 ± 0.26 % in 1 and 2 mg/disc, respectively against *S. sonnei*. Petroleum ether, n-butanol and aqueous extracted samples showed no activity against *Citrobacter* sp. (fig. 2A). Crude methanol extract reduced the growth of the same bacterium by 23.33 ± 0.50 and 33.33 ± 0.43 % in 1 and 2 mg/disc concentrations, respectively. Ethyl acetate extract showed activity of 33.33 ± 0.40 and 36.70 ± 0.40 % at concentrations of 1 and 2 mg/disc, respectively against *Citrobacter* sp.

Crude methanol, petroleum ether, chloroform, ethyl acetate, n-butanol and aqueous extracts were also tested

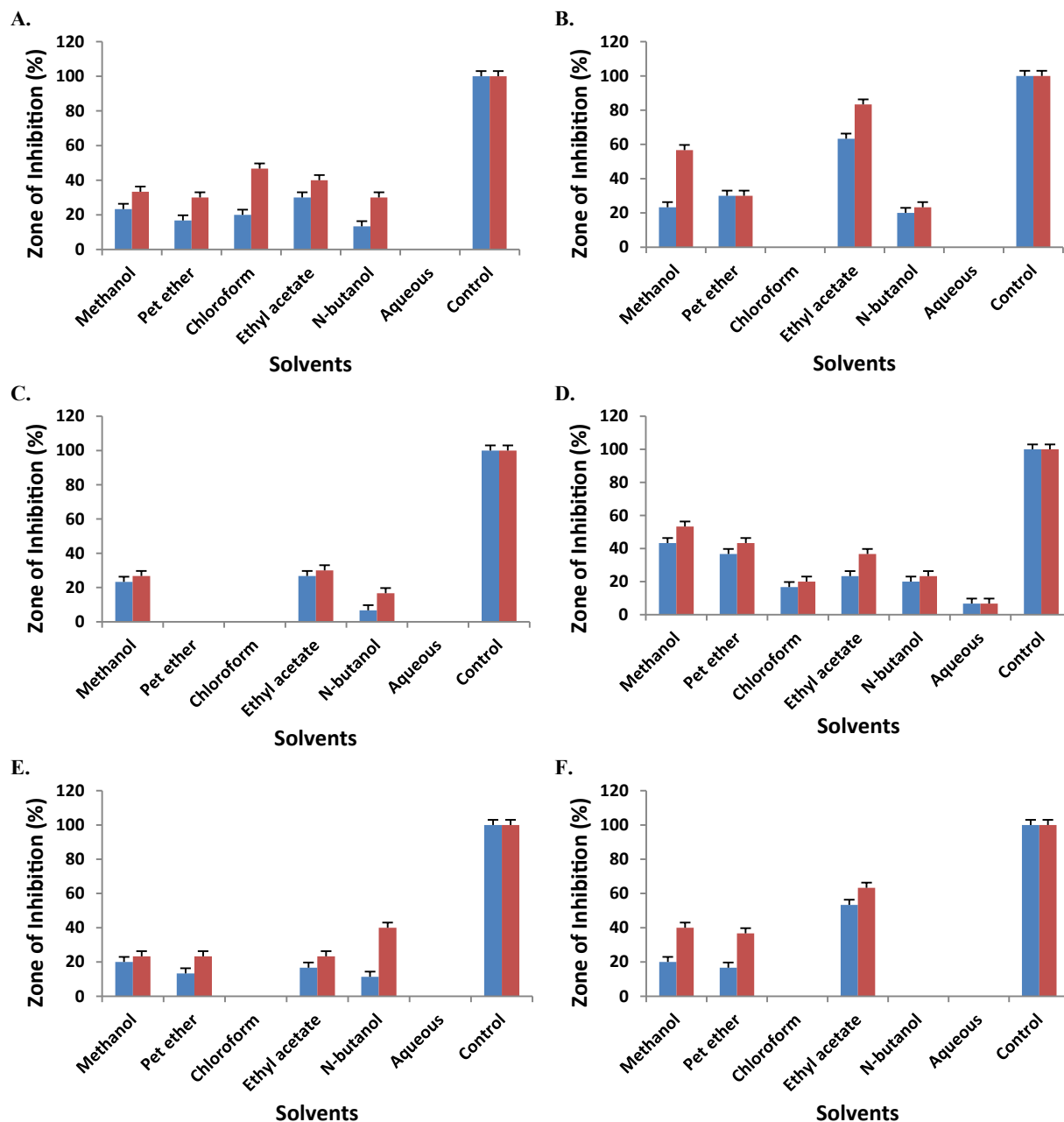


Fig. 1: Antibacterial activity of different extracts of *Chrozophora tinctoria* against (A) *Escherichia coli*, (B) *Salmonella typhi*, (C) *Pseudomonas aeruginosa*, (D) *Providencia sp.*, (E) *Proteus mirabilis* and (F) *Shigella sonnei*, using disc diffusion assay. Bar shows LSD value at $p < 0.05$. ■ 1 mg/disc; ■ 2 mg/disc

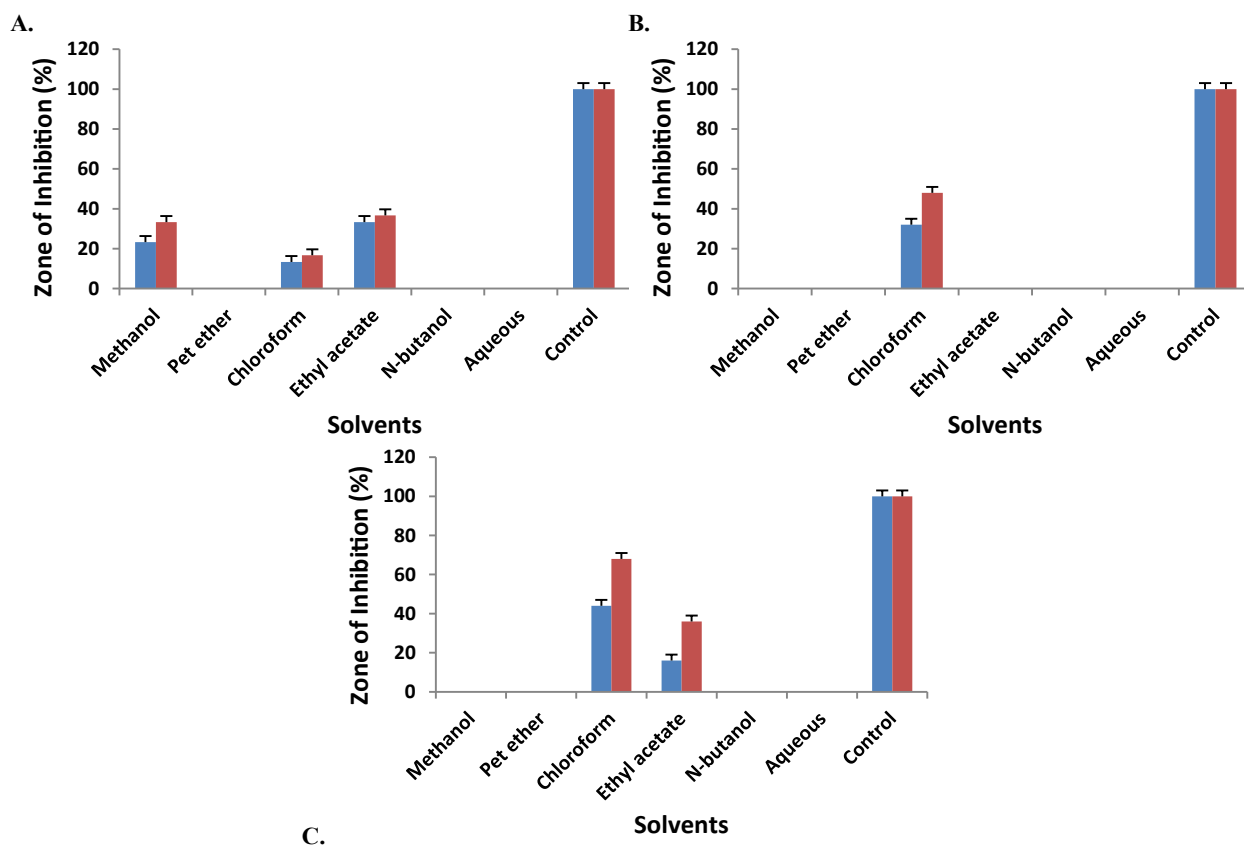


Fig. 2: Antibacterial activity of different extracts of *Chrozophora tinctoria* against *Citrobacter sp.*, *Aspergillus fumigatus* and *Rhizopus sp.* using disc diffusion assay. Bar shows LSD value at $p < 0.05$. ■ 1 mg/disc; ■ 2 mg/disc

against *Aspergillus fumigatus* as shown in fig. 2B. Almost all the extracts showed no activity against *A. fumigatus* at both concentrations except for chloroform extract, which showed 32 ± 0.34 % inhibitory activity at 1 mg/disc and 48 ± 0.20 % inhibitory activity at 2 mg/disc. All the extracts of *C. tinctoria* did not reduce the activity of *Penicillium chrysogenum* at both concentrations showing no antifungal activity. Most of the extracts showed no zones of inhibition against *Rhizopus sp.* except chloroform and ethyl acetate extracts (fig. 2C). Chloroform extract inhibited bacterial growth by 44 ± 0.17 and 68 ± 0.45 % at 1 and 2 mg/disc concentrations, respectively. Similarly, ethyl acetate extract produced 16 ± 0.17 and 36 ± 0.30 % inhibition at 1 and 2 mg/disc, respectively (fig. 2C).

Hot plate method is more sensitive to centrally acting analgesics. Thermal-induced nociception indicates narcotic involvement^[21]. The methanol extract of *C. tinctoria* significantly ($p < 0.05$) and dose-dependently protected the mice against thermally induced pain stimulus. However, the extract at 150 mg/kg showed increased latency time. Magaji *et al.*^[22]

reported comparatively similar results in *Securinega virosa* (Euphorbiaceae) where the methanol extract of the root bark at 12.5 mg/kg concentration showed significant ($p < 0.05$) mean latency period of 14.20 ± 1.61 s and at higher concentration of 25 mg/kg the mean reaction time increased to 17.60 ± 1.08 s. AA-induced method was used for identifying peripheral analgesia. Crude methanol extract significantly ($p < 0.05$) affected the AA-induced abdominal writhes in mice. Crude methanol extract resulted in the highest inhibition of abdominal constriction when given at 150 mg/kg. Crude methanol extracts inhibited 50 % of the abdominal constriction and proved to be 50 % antinociceptive at 150 mg/kg. Jigam *et al.*^[23] reported that the extract of *Curvularia senegalensis* inhibited abdominal constriction.

Crude methanol extracts of *C. tinctoria* at 100 mg/kg showed significant ($p < 0.05$) effects on antipyretic activity. A decline in temperature occurred up to $100.55 \pm 0.39^\circ$ F and $100.2 \pm 0.309^\circ$ F, respectively as compared to control (normal saline) group after 3 and 4 h. The extracts in 200 mg/kg dose revealed significant

($p < 0.05$) effects after 1, 2, 3 and 4 h. A gradual fall in temperature occurred after 1 h and onward as compared with normal saline group. Similarly, extracts in 300 mg dose also revealed significant ($p < 0.05$) antipyretic activity. Increasing quantity of the extracts measured higher antipyretic activity. These results showed similarities to *Euphorbia hirta* belonging to family Euphorbiaceae where antipyretic activity was obtained at the sedative doses of 100 and 400 mg/kg on the yeast-induced hyperthermia^[24].

Most of the extracts of *C. tinctoria* were effective against the tested bacterial strains except aqueous extract where no activity at both concentrations was observed. Similar results are also reported by Igbiosa *et al.*^[25] who concluded that water extracts from the stem bark of *Jatropha curcas* (Euphorbiaceae) showed low antibacterial activity with inhibition zones ranging between 0 and 8 mm (0-26.67 %) for different tested bacteria. Among different solvent extracted samples, crude methanol and ethyl acetate extracts showed activity above 50 % against *S. typhi* and *Providencia* sp. and above 30 % against *E. coli*, *S. sonnei* and *Citrobacter* sp. in highest concentration. Similarly, ethyl acetate extract was also effective against most human pathogens showing above 60 % activity against *S. typhi* and *S. sonnei* in concentration of 2 mg/disc and above 50 % in concentration of 1 mg/disc. Ethyl acetate extract reduced the growth of *E. coli*, *Providencia* sp. and *Citrobacter* sp. above 30 %. These results agree with Nelofar *et al.*^[26] who revealed that ethyl acetate extract was effective against Gram-positive and Gram-negative bacteria. Similarly, Usman *et al.*^[27] reported that ethyl acetate extract of *C. senegalensis* was effective to reduce the growth of *Bacillus subtilis*, *E. coli*, *P. aeruginosa*, *Staphylococcus aureus*, *S. typhi* and one fungus *Aspergillus flavus*. *C. tinctoria* extracts were also tested against *A. fumigatus*, *P. chrysogenum* and *Rhizopus* sp., but produced no activity against the microorganisms tested except the chloroform extract.

It could be concluded from these results that *C. tinctoria* extract possessed analgesic activity when given at a dose of 150 mg/kg, inhibiting the abdominal constriction to 50 %. The antipyretic effect of crude methanol extract at a dose of 300 mg/kg revealed significant decrease in body temperature of mice. Different extracts were effective to reduce the growth of different strains of bacteria and fungi.

Conflict of interest:

The authors declare no conflict of interest.

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