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COMPARATIVE ANTIBACTERIAL, CYTOTOXIC, ANTI-INFLAMMATORY AND HEPATOPROTECTIVE ACTIVITIES OF CAPSULES, LEAVES AND BARK OF *CASEARIA TOMENTOSA* ROXB.

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ABSTRACT

The antibacterial, anti-inflammatory, cytotoxic and hepato-protective potential of the leaves, bark and capsule of *Casearia tomentosa* Roxb. of family Flacourtiaceae was evaluated to investigate their Ethnopharmacological importance. *C. tomentosa* bark and fruit (capsule) extracts antimicrobial potential was analyzed through Agar well diffusion method against microbes such as *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*. Activity represents strong potential of capsule extract at 200 mg concentration against *B. subtilis*, *K. pneumoniae* and *A. baumannii* with zone of inhibition 24 ± 1.49mm, 23 ± 2mm and 17.33 ± 1.52mm respectively while poor potential against *E. coli*. Despite that the 50 mg concentration of capsule extract showed strong activity with 20.66 ± 1.52 mm zone of inhibition against *E. coli. C. tomentosa* Roxb. bark extract showed moderate activity with 200 mg concentration against *B. subtilis* and *K. pneumoniae* with zone of inhibition 17.33 ± 0.58 and 17 ± 1 respectively. While it showed no activity at 50 mg concentration towards any bacteria.

Carrageenan-induced paw edema method is assisted to determine capsule anti-inflammatory abilities. Oral administration of capsule extract with concentration of 200 mg/kg and Four hundred mg/kg of body weight in mice were subjected, accompanying piroxicam in positive control group. 400 mg/ kg dose exhibited significant results as compared to 200 mg/kg with respect to positive control against inflammation.

C. tomentosa capsule extract hepatoprotective activity was analyzed with carbon tetrachloride induced liver injury in mice with individual dose of 2 mL/kg p.o. with the 1:1 combination of olive oil. Doses of 200 and 400 mg/kg b.w. were applied for 21 days while CCl_4 of about (1mL/kg) by subcutaneous route after every 72 h. Serum enzymatic levels such as SGOT, SGPT and ALP represent the efficacy of 400 mg/kg over 200 mg/kg dose of extract against hepatotoxicity.

Keywords: *Casearia tomentosa*, antibacterial, anti-inflammatory, cytotoxic and hepato-protective.

INTRODUCTION

Plants are considered to be the most ancient healing source against variety of human diseases. Moreover, their secondary chemicals are the wealth sources of important modern drugs named as atropine, codeine, digoxin, morphine, quinine and vincristine etc. (Angle and Sardessai, 2019).

Phytochemicals are well known for their protective and curing behaviour against aliments. Plants produce these chemicals for their defensive purposes but advance studies reveal their strong responses against different ailments (Mentor *et al.,* 2014). Plant extracts have been used enormously with and without chemical modification for various infectious diseases. Investigation of plant extracts regarding their antimicrobial activity has shown that they represent potential for new anti-infective compounds (Louis *et al.,* 2018).

Antimicrobial activity is the practice to kill or control or inhibit the microbes specifically behind the respective disease. Prior and recent studies have compiled the wide beneficial aspects of the plants extracts against microbes, bacteria and fungi activities using different cultures and techniques (Joseph and Raj, 2010). A renowned proportion of plants suggested to possess rich yield of antimicrobial compounds, and thus, it is essential requirement to screen flora for the more advance, efficient and eco-friendly microbe killers (Gideon *et al.,* 2017).

The present era is facing number of serious health problems; inflammation is one of them. The anti-inflammatory drugs act as healing and curing agents against many diseases such as arthritis and rheumatic fever, hence called to be lifesaving agents. Clinically anti-inflammatory drugs acquire significant position due to their strong effect on the pain and stiffness (Vishal *et al.,* 2014). Inflammation is commonly known as reaction against injury, disease or disturbance of living tissues (Kumar *et al.,* 2013).

Secondary metabolites or chemical constituents of various medicinal plants found to be effective against inflammation and pain. The ethnobotanical investigation of plants reveals their utilization as pain relievers that has gain attention for their anti-inflammatory effect (Amri *et al.,* 2018).

The liver is the key organ related with body homeostasis (Sharma and Sharma, 2010). Synthetic substances that give rise to liver injury are called hepatotoxins. More than 1000 medications as appear to be causing liver injury and it is the principal essential purpose behind a few medications to be pulled back from the market (Khan *et al.,* 2018).

The Brine shrimp lethality measure a fast, modest and straightforward bioassay for testing plants extricate bioactivity which relates sensibly well with cytotoxic and antitumor properties (Asba and Meeta, 2017).

Casearia tomentosa Roxb. recognized as member of coffee plum family i.e. Flacourtiaceae embodies 90 genera surrounded and 1,000 species. Among them 3 genera with 5 species were found for in Pakistan. *C. tomentosa* is vernacularly known as Chilla (Fig. 1). It is a small to medium size tree (up to 7 m) seen in the uneven beds of Pakistan to India, Malaysia and North Australia (Ajaib *et al.,* 2015). Leaves are elliptic, toothed, oblong or ovate and narrowed towards the tip. Stem is 5-12cm long, Stalk 6-12 mm long, midrib and stalk of full grown leaves are hairy. *C. tomentosa* fruit are capsule, ellipsoid, dehiscent, 6-ribbed, 3-valved, with seeds embedded in a red, scarlet pulp. Flowers are greenish, yellow in dense axillary clusters. Fruits are capsule, ellipsoid, with scarlet pulp (Ajaib 2012; Malleswari *et al.,* 2015).

C. tomentosa has a wide scope of therapeutic employment. Each part of the plant is useful in various fields with appreciable effects. Root bark is reputed as a stimulant in anaemic conditions. Plant root and bark decoction after a span of boiling; collected by sieving from plant parts, consumed to treat diabetes. Latex of the bark is also externally applicable for the treatment of snake bite. Powdered bark is applied externally in dropsy and fever (Tyagi *et al.,* 2017).

The leaves are employed in medicinal baths and their leaf paste is used as anthelmintic. The fruit pulp is a useful diuretic. The juice from the fruit is used as a fish poison. Oil extracted from the seeds is rubbed on sprains. Seeds are steeped in hot water afterwards applied on the swellings to promote suppuration and to hasten the eviction of worms (Angle and Sardessai, 2019).

MATERIALS AND METHODS

Plant Collection and extraction

The plant *Casearia tomentosa* Roxb. was collected from Samahni Azad Kashmir, Pakistan in September 2019 (Fig. 1). The plant was identified from herbarium Department of Botany, Mirpur University of Science and Technology with voucher specimen number MUH-652.

Capsule, leaves and bark powder of *C. tomentosa* (250 g) was weighed accurately by electrical balance and drenched in ethanol separately. The soaked plant material was then filtered by Whatman filter paper after 7 days. The residue of every respective plant part was again drenched in ethanol for another 7 days by and sifted with Whatman filter paper. Repeated the respective process for another 7 days and filtered. The residue was discarded and extract was concentrated on a rotary evaporator.

Fig. 1. *C. tomentosa* Roxb.

Antimicrobial activity
Agar well diffusion method

Agar-well diffusion technique was performed for estimation of inhibitory zone (Pingale *et al.,* 2019). The autoclaved nutrient agar medium was prepared with the 1917, American Public Health Association standard. The semi-solid extracts of bark of *C. tomentosa* and capsule were organized after maceration. Ethanolic 50 mg and 200 mg corresponding amount of macerates were taken into separate glass vials and drenched with 2 mL of ethanol. The extracts were thoroughly dissolved into solvents and glass vials stored at 20℃. About 20 mL autoclaved nutrient agar poured into autoclaved Petri plates. The inoculum was prepared using broth medium for *Bacillus subtilis, Escherichia coli, Acinetobacter baumannii, Klebsiella pneumoniae* strains (Table 1). The inoculum was extended thoroughly using sterile cotton mop on solidified medium gently. Four wells were set up with the assistance backing of plug drill no. 4 in the parallel crossed positioned with 1 well at the center of the Petri plate. From the 50 mg bark and capsule extracts of *C. tomentosa* concentration dilution, 30µL and 60µL of volume were replenished the crossed wells while about 30 µL volume of ethanol was subjected to the central hole of Petri plate. Beside that from 200 mg bark and capsule extracts of *C. tomentosa* concentration dilutions, 80µL and 120µL of volume were subjected to the corresponding wells with 50 µL of ethanol in the central well with the help of micropipette and put for incubation at 37 ± 2 ℃ for the span of 24 h. after that's zone was measured in mm.

Anti-inflammatory activity

Carrageenan-Induced Paw edema in Mice

C. tomentosa capsule extract anti-inflammatory was conducted on Twenty mature male Swiss albino mice ranges in weigh to 25-30 g were selected. With due permission of ethical committee of Government College University, Lahore. Mice were kept for a week under standard conditions i.e. taken care with pelleted diet and water promotion libitum, with 12 hours' dark and 12 h light rhythm, temperature: 25-27 ℃ and humidity 55-57 % constantly before initiation of the experiment. *C. tomentosa* ethanolic capsule doses of 200 mg/kg and 400mg/kg was stabilized by diluting it in olive oil. The mice were distributed into four gatherings containing five creatures in every one. Group I was alluded as negative control. Group II was subjected 200mg / kg capsule extract orally. Group III received 400mg / kg capsule extract per orally whereas Group IV was referred as positive control. Administrated with piroxicam at a portion of 10 mg / kg per orally.

Thirty minutes later of oral dose in all groups, 50 µL of sterile saline suspension of 1% w/v carrageenan was infused into the sub-plantar surface of the left rear paw. Paw size was estimated utilizing Vernier caliper at time 0, 1, 2, 3, 4 h (Table 2) after the carrageenan organization following Bairagi *et al.,* (2017).

Hepatoprotective activity

CCl_4 Induced Liver Injury

C. tomentosa capsule extract hepatoprotective potential was determined on 28 mature male Swiss albino mice ranging in weight from 25 to 30 g. With due permission of ethical committee of Government College University, Lahore; mice were housed in the animal house of Zoology department, for experimentation purpose. Mice were kept for a week under standard conditions i.e. taken care with pelleted diet and water promotion libitum, with 12 h dark and 12 h light rhythm, temperature: 25-27 ℃ and humidity 55-57 % constantly before initiation of the experiment. The relative doses of ethanolic capsule extract of *Casearia tomentosa* were designed by saturated it in olive oil while Carbon Tetrachloride was set up by incorporation with olive oil in the relative ratio of 1:1. Animals were alienated into 4 categories each with 7 animals. Group I was designated on Olive oil of about 2ml/kg per oral on daily basis throughout the investigation (Fig. 3). The group was oppressed as negative control. Group II animals get olive oil throughout the experiment and CCl_4 (Fig. 4) of about (1mL/kg) by subcutaneous route after every 72 h. The group was categorized as positive control. Group III (Fig. 5) received Capsule extract of 200mg/kg body weight p.o. on daily basis. After every 72 h. concurrently administered CCl_4 by subcutaneous route parallel to extract dose. Group IV (Fig. 6) received Capsule extract of 400mg/kg body weight p.o. on daily basis. After every 72 h. concurrently administered CCl_4 by subcutaneous route parallel to extract dose.

Animals were treated in respected manner for a period of 21 days. At the end of every 72 h i.e.$4th$, $7th$, $10th$, $13th$, $16th$, $19th$ & $21st$ day CCl_4 was managed to all gatherings with the exception of gathering I. The mice were kept up under ordinary eating routine and water all through the time of treatment as per followed by Janarthan (2014).

On $21st$ day of experimentation, after subjected to the last dose of $CCl4$ animals were sacrificed after time span of 24 h. under mild anesthesia. Blood sample were collected from heart of animals from all groups utilizing dispensable needle. Blood was permitted to cluster at room temperature for 30 min. and afterward exposed to centrifugation (4000 rpm for 15 min.) lastly serum is gathered for biochemical inspections of SGOT, SGPT and ALP (Fig. 2). After blood collection of an animal, simultaneously liver tissues were excised. Tissues were washed

thoroughly with ice-cold normal saline. At that point parts of tissues were put in 10% formalin solution for histopathological study.

Cytotoxicity

Brine Shrimp Lethality Assay (BSLA)

Brine shrimps were hatched by utilizing 2 liters of distilled water mixed thoroughly with 27 g of table salt and filtered. The salt water is kept into a rectangular jar portioned to two halves with three small holes in separating sheet. 1 g of Brine shrimp eggs were added to salt water and covered while the other half illuminated with light to keep the hatching temperature maintained. The hatch nauplii move toward the second portion of jar due to their photo tactic behaviour. Hence easily collected and monitored as needed. The nauplii were hatched after 24 h. and were collected after next 2 h**.** Stock Solutions of leaves, bark and capsule extracts of *C. tomentosa* was prepared by dissolving 20 mg in 1 mL of solvent (ethanol) independently while serial dilutions of 1000 µg/mL, 100 µg/mL, 10 µg/mL and 1 µg/mL were prepared for all three extracts individually. Ethanol with salt water was considered as negative control group. Phenol with salt water is taken as positive stand for the experiment.

Triplicates for 1mL serial dilution of each extract were set and by utilizing Pasteur pipette and 10 nauplii were introduced into each test tube with the help of micropipette. Similar numbers of nauplii were exposed to positive and negative standard test tubes triplicates and finally counted the number of dead larva after 24 h.

Statistical calculations

For all the activities the readings were carried out in triplicates and data was arranged as mean value ± S.E using Microsoft excels. Probits analysis and linear correlation was fitted in sense of graphs in cytotoxicity assay.

RESULTS

Antibacterial activity

The zone showed by extracts of bark and capsule of *C. tomentosa* was measured in millimeter. Ethanol used during maceration was referred as negative control.

Table 1. Inhibitory zone (mm) shown by bark and capsule extract of *C. tomentosa* against microbes.

Plant part extract	**Conc. (mg)**	**V. (µL)**	**Solvent**	**Inhibitory Zone (mm)**			
				B. subtilis	***K. pneumonia***	***A. baumannii***	***E. coli.***
Capsule	50	30	Ethanol	14.32 ± 1.52	9 ± 1	8.33 ± 1.15	14.33 ± 1.56
		60		18 ± 1	11 ± 1	9 ± 1.73	20.66 ± 1.52
	200	80		22.33 ± 1.52	21 ± 1	17.33 ± 1.52	8.43 ± 1.52
		120		24 ± 1.49	23 ± 2	14.66 ± 0.81	6.63 ± 1
Bark	50	30		10.33 ± 3.05	7 ± 1	6.33 ± 0.57	7 ± 1
		60		11 ± 1	10 ± 2	7.66 ± 15.2	19 ± 1
	200	80		17.33 ± 0.58	7.43 ± 1.52	9 ± 1	8.34 ± 0.57
		120		13.64 ± 1.52	17 ± 1	7 ± 1.73	7.68 ± 1.15

Anti-inflammatory

The reaction of treatment groups was compared with group received that standard drug Piroxicam. 400mg/ kg ethanolic extract significantly reduced swelling which is near to standard drug Piroxicam in Table 2.

Table 2: *C. tomentosa* capsule extract effect on carrageenan-induced paw edema.

Plant part	**Gatherings**	**Mean Paw Diameter (mm)**				
		0 h	**1 h**	**2 h**	**3 h**	**4 h**
***C. tomentosa* capsule**	**Negative control**	5.9 ± 0.08	6.8 ± 0.21	6.6 ± 0.25	6.36 ± 0.36	6.16 ± 0.19
	200 mg Plant extract	6.1 ± 0.21	6.7 ± 0.09	6.5 ± 0.17	6.2 ± 0.14	5.9 ± 0.13
	400 mg Plant extract	6 ± 0.1	6.3 ± 0.2	5.7 ± 0.22	5.37 ± 0.2	4.8 ± 0.08
	Piroxicam	6.14 ± 0.15	5.68 ± 0.2	4.62 ± 0.2	4.06 ± 0.21	3.52 ± 0.11

Hepatoprotective potential

For CCl_4administrated hepatotoxicity study in mice the actions of SGOT, SGPT & ALP serum hepatic marker enzymes were carried out. The 400mg/kg capsule extract showed significant activity in all three parameters.

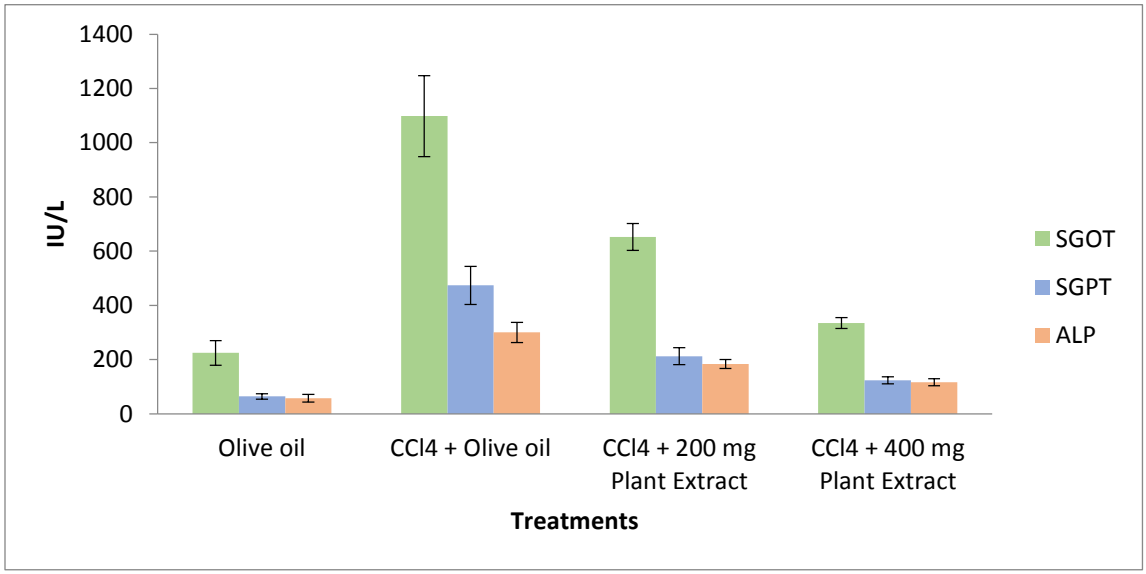

Fig. 2. Measurement of SGOT, SGPT and ALP in all treatments for hepatotoxicity.

Histopathological studies

The slides of lives tissues of all groups competing in hepatoprotective activity showed the significant impact of treatments subjected to them.

(A) **Olive oil control group:** Architecture of normal liver (olive oil control group) (Fig. 3).

(B) $\boldsymbol{CCl_4}$**+ Olive oil:** the liver is damaged which is evident by distortion of hepatocytes integrity, spillage of lymphocytes and loss of cellular boundaries (Fig. 4).

(C) $\boldsymbol{CCl_4}$**+ 200 mg Capsule Extract:** The plants showed hepatoprotective effect as the hepatocytes architecture is comparable to that of olive oil control (Fig. 5**)**.

(D)$\boldsymbol{CCl_4}$**+ 400 mg Capsule Extract:** The plant exhibited hepatoprotective activity as the liver architecture reverted to the normal (Fig. 6).H & E (40x magnification).

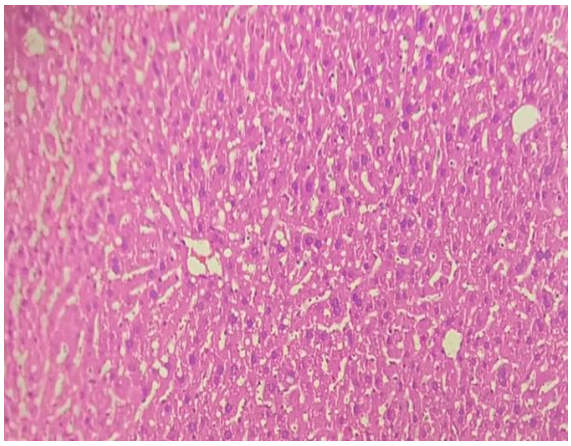

Fig. 3. Liver section of control group (olive oil)

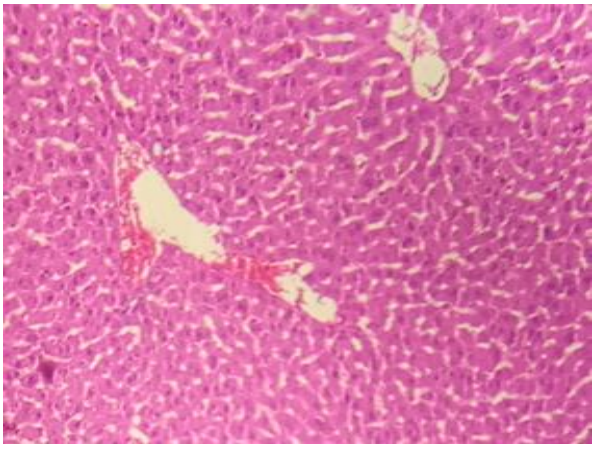

Fig. 4. Liver section of CCl_4+ Olive oil treatment.

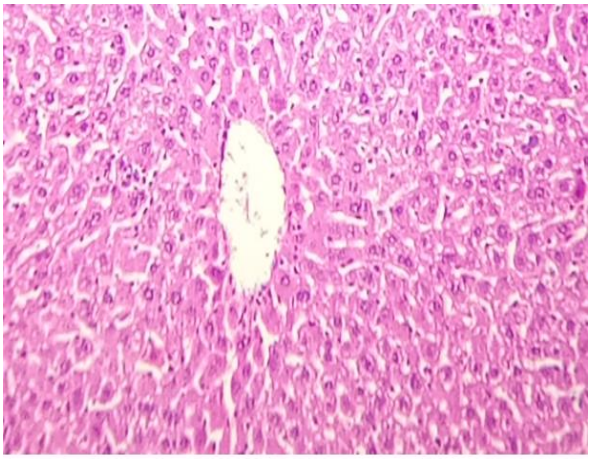

Fig. 5. Liver section of CCl_4+ 200 mg Capsule extract treatment group,

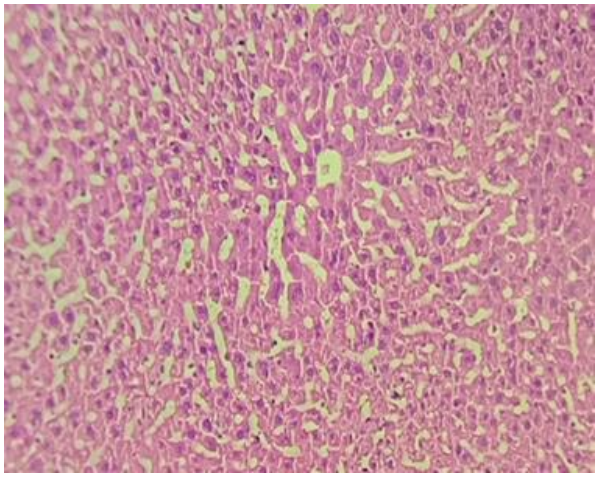

Fig. 6. Liver section of CCl_4+ 400 mg Capsule extract treatment group,

Table 3. Average death of *Artemia salina* at different concentration of ethanolic crude extract of *C. tomentosa* leaves, bark and capsule extract.

***C. tomentosa* crude extract**	**Average death of *Artemia salina***				**LC_{50}**
	Concentrations (µg/mL)				
	1000 µg/mL	**100 µg/mL**	**10 µg/mL**	**1µg/mL**	
Leaves	7.33 ± 1.20	5.33 ± 0.88	4.33 ± 0.67	2.33 ± 1.33	28.24
Bark	5.33 ± 0.33	3.66 ± 0.66	1.66 ± 0.33	1.33 ± 0.33	810
Capsule	8 ± 0.57	6.33 ± 0.66	5 ± 0.57	3.66 ± 0.33	9.65
Negative control	0	0	0	0	0
Positive control	10 ± 0.00	10 ± 0.00	9.66 ± 0.33	10 ± .00	1.49

Cytotoxicity

Mortality

The percentage mean mortality (%M) was also calculated by following formula

$$\% \text{ Mortality} = \frac{\text{Number of dead nauplii}}{\text{Number of total nauplii}} \times 100$$

DISCUSSION

The antibacterial activity was performed to analyzed the bark and capsule extract potential against various microbes because from the last two decades, there has been a spectacular rise to find novel drugs and agents from natural products which offers strong potential against antimicrobial activity with lesser side effects towards receiver.

The activity was done with simple, rapid and cost-effective agar well diffusion method for assessment of the inhibitory zone against bacterial strains. The inhibitory zones presented by the plant bark and capsule ethanolic macerates against the test microbes were alike with the findings achieved by Angle and Sardessai, (2019) while studied impact of solvent types on antimicrobial activities of root extract of *Casearia tomentosa*.

The anti-inflammatory effect shown by plant was due to presence of flavonoids and terpenoids. Flavonoids are placed among phenolic compounds that possessed antimicrobial, antioxidant, anti-inflammatory, anticancer activities as quoted by Okwu, (2001) in its finding of evaluation of the chemical composition of indigenous spices and flavoring agents. Whereas Wang *et al.,* (2002) reported terpenoids to be most prevalent diverse groups of biochemical active components which bear potential against cytotoxic, anti-inflammatory activities in its article Phytosterols, triterpene alcohols, and phospholipids in seed oil from white lupin.

Hepatoprotective nature of *C. tomentosa* capsule was analyzed by CCl_4induced hepatotoxicity in mice through 200mg/kg and 400 mg/kg doses (Fig. 5 & 6). The control group animals are only served with olive oil similarly as Heibatollah *et al.,* (2008) while studied hepatoprotective effect of *Cichorium intybus* on CCl_4- induced liver damage in rats.$CCl_4$was applied as a 1:1 ratio with olive oil (1mL/kg) intraperitoneally after every 72 h. The results are evaluated by measuring ALP, SGPT and SGOT levels (Fig. 2). The most significant results were shown by 400 mg/kg in all of the three serum levels ALP, SGPT AND SGOT i.e. 116.5 ± 5.22, 123.12 ± 4.94 and 335 ± 7.55 respectively. While 200mg/kg extract revealed favorable decrease in ALP and SGPT serum levels such as 183.62 ± 6.32 and 212.5 ± 11.80 respectively. The given results are parallel to the hepatoprotective functioning recorded by Aniya *et al.,* (2005) while evaluating hepatoprotective actions of the medicinal herb, *Crassocephalum crepidioides* from the Okinawa Islands.

The ethanolic crude extract of leaves, bark and capsule of *C. tomentosa* was tested for their toxicity with help of Brine Shrimp Lethality Assay (BSLA) (Table 3). Stock solutions were prepared with respect to 1000 µg/mL, 100 µg/mL, 10 µg/mL and 1 µg/mL concentrations as also studied by Sharififar *et al.,* (2017) in bioassay screening of the essential oil and various extracts of *Nigella sativa* L. seeds using Brine Shrimp Toxicity Assay. All the concentrations were practiced with three triplicates containing 10 shrimps in each. *C. tomentosa* leaves extract shows mean mortality 73.3 %, 53.3%, 46.6% and 30%, besides average death of *Artemia salina* 7.33 ± 1.20, 5.33 ± 0.88, 4.33 ± 0.67 and 2.33 ± 1.33 with the concentrations of 1000 µg/mL, 100 µg/mL, 10 µg/mL and 1 µg/mL respectively was similar with findings of Dokuparthi *et al.,* (2018) while studied brine shrimp lethality bioassay of *Bougainvillea glabra*. On the other hand, *C. tomentosa* bark extract showed good toxicity at 1000 µg/ml concentration with 53.3 % mortality and 5.33 ± 0.33 average shrimps death. Whereas weak lethality of about 36.6%, 16.6% and 13.3% with 100 µg/ml, 10 µg/ml and 1 µg/ml respectively. Ethanolic capsule extract of experimented plant gives strong toxicity as compare to leaves and bark. 80% mortality with 8±0.57 average death was seen with 1000 µg/ml concentration, followed by 63.3% mortality and 6.33±0.66 average animal death with 100 µg/ml concentration. Both 10 µg/ml and 1 µg/ml confirms moderate lethality as compare to others, former bearing mortality of 50% and latter one with 36.6 % comprising average death of *Artemia salina* at 5±0.57 and 3.66±0.33 respectively.

CONCLUSION

The man kind was directly relying upon nature utilizing plants as source of food, shelter and particular agents against numerous ailments and infections. From centuries plants assists human life in form of plant-based natural products as well-off source of modern medicines. Leaves and capsule of plant exhibit appreciable anti-bacterial potential against both gram-negative and positive bacteria i.e. *B. subtilis* and *A. baumannii, K. pneumoniae* and *E. coli* respectively. While the *C. tomentosa* bark is effective against *B. subtilis* (gram-positive bacterium). Ethanolic capsule bears significant anti-inflammatory and hepatoprotective potential concluded with in-vivo carrageenan paw induced edema and CCl_4 induced hepatotoxicity in mice respectively. Cytotoxicity of investigated plant parts were experimented with Brine Shrimp Lethality Assay which confirms the rich possession of lethality in Leaves and

capsule whereas weak in *C. tomentosa* bark. Further investigations would be required to explore the plant Ethnopharmacological prospective.

REFERENCES

Ajaib, M. (2012). Exploration of floral diversity of district Kotli (Azad Jammu & Kashmir) and evaluation of ethnopharmacological effects of some medicinal plants of the area. Ph.D. thesis, Department of Botany, GC University, Lahore, Pakistan.

Ajaib, M., S. Khalid, K. M. Khan, S. Z. Siddiqui, M. A. Abbasi, S. Perveen and S. Shah (2015). *Casearia tomentosa*: A Potential Antimicrobial and Antioxidant Source. *J. Chem. Soc. Pak.,* 37 (4): 811-816.

Amri, O., A. Zekhnini, A. Bouhaimi, S. Tahrouch and A. Hatimi (2018). Anti-inflammatory Activity of Methanolic Extract from *Pistacia atlantica* Desf. Leaves. *Pharmacogn. J.,* 10 (1): 71-76.

Angle, G. P. and Y. Sardessai (2019). Impact of Solvent Types on Antimicrobial Activities of Root Extract of *Casearia tomentosa*. *Int. J. Pharm. Pharm. Sci.,* 11 (4): 33-38.

Aniya, Y., T. Koyama, C. Miyagi, M. Miyahira, C. Inomata, S. Kinoshita, T. Ichiba (2005). Free radical scavenging and hepatoprotective actions of the medicinal herb, Crassocephalum crepidioides from the Okinawa Islands. *Biol. Pharm. Bull.,* 28: 19-23.

Asba, A., B. Meeta (2017). Evaluation of Phytochemicals of *Cassia tora* Linn. and its Cytotoxicity Assay using Brine Shrimp. *Int. J. Pharmacogn. Phytochem. Res.,* 9 (4): 587-595.

Bairagi, S.M., I. B. Pathan and N. Nema (2017). Analgesic and anti-inflammatory activity of crude leaf and bark extract of *Lantana Camara*. *Marmara. Pharm. J.,* 21 (4): 810-817.

Dokuparthi, S.K., G. Lakshmi, A. Anjana, S. F. Fatima, S. Ashwini, S. Kandagatla and S. Raj (2018). Brine shrimp lethality bioassay of *Bougainvillea glabra*. J. *Drug Delivery & Therapeutics*, 8 (4): 244-246.

Gideon, P.N., J. M. Nzei, J. M. Munywoki, H. Louis, F. G. Kwata and T. Fidelis (2017). Phytodiversity, abundance and importance of some useful plants at Mangrove swamp forest of Sobekiri rivers state Nigeria. *Int. J. Sci. Res.,* 7 (3): 327-331.

Heibatollah, S., N. M. Reza, G. Izadpanah and S. Sohailla (2008). Hepatoprotective effect of *Cichorium intybus* on CCl4- induced liver damage in rats. *Afr. J. Biochem. Res.,* 2(6): 141-144.

Janarthan, A. (2014). Evaluation of hepatoprotective activity of *Elaeocarpus ganitrus* leaf extract against CCl4 induced liver damage. *J. Chem. Pharm. Sci.,* 7 (4).

Joseph, B. and S. J. Raj (2010). Phytopharmacological and phytochemical properties of three *Ficus* species-an overview. *Int. J. Pharma. Bio. Sci.,* 1 (4): 246-53.

Khan, F. S., M. Akram, N. Aslam, J. Zaheer, S. B. Mustafa, S. Kausar, A. H. Khan, I. A. Khan, N. Munir, S. M. A. Shah, I. M. Tahir and A. Sharif (2018). Phytochemical analysis and hepatoprotective effect of polyherbal formulation on CCl4 induced hepatotoxicity in mice. *Pak. J. Pharm. Sci.,* 31 (6): 2719-2723.

Kumar, B. S. A., G. S. Saran, A. Mouna and C. N. Kumar (2013). *In Vitro* Anti–Inflammatory Activity of *Tankana churna*. *Food Feed Res.,* 40 (1): 17-20.

Louis, H., M. N. Linus, A. Israt, J. Innocent, P. I. Amos and T. O. Magu (2018). Antimicrobial Activity of Stem, Leave and Root Plant Extract of *Sclerocarya birrea* and *Sterculia setigera* against Some Selected Microorganisms. *World Sci. News,* 92 (2): 309-326.

Malleswari, M., G. karra, U. Maheswarao, V. R. Chidrawar (2015). Alleviating Effect of Leaf Extract of *Casearia Elliptica* Against Paracetamol and Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats. *Int. J. Uni. Pharm. Bio Sci.,* 4 (6): 157-242.

Mentor, R., H. B. Jovanova, T. K. Panovska (2014). Toxicological evaluation of the plant products using Brine Shrimp (*Artemia salina* L.) model. *Maced. pharm. Bull,* 60 (1): 9-18.

Okwu, D.E. (2001). Evaluation of the chemical composition of indigenous spices and flavouring agents. *Global I. Pure Appl. Sci.,* 7: 455-9.

Pingale, T., P. Duse and S. Ogale (2019). Antibacterial and antifungal Approaches of *Ficus racemosa*. *Pharmacogn. J.,* 11 (2): 355-357.

Sharififar, F., A. Assadipour, M. H. Moshafi, F. Alishahi and H. Mahmoudvand (2017). Bioassay screening of the essential oil and various extracts of *Nigella sativa* L. seeds using Brine Shrimp Toxicity Assay. *Herb. Med. J.,* 2 (1): 26-31.

Sharma, B., U. K. Sharma (2010). Hepatoprotective activity of some indigenous plants. *Int. J. Pharma. Tech. Res.,* 2 (1): 568-572.

Tyagi, R., A. Shukla and R. K. Shukla (2017). Phytochemical screening and pharmacological evaluation of different extracts of plant *Casearia tomentosa* leaves. *Int. J. Pharm. Pharm. Sci.,* 9: 166-71.

Vishal, V., S. N. Ganesh, G. Mukesh and B. Ranjan (2014). A review on some plants having anti-inflammatory activity. *Int. J. Pytopharm.,* 3 (3): 214-221.

Wang, T., K. B. Hicks and R. Moreau (2002). Phytosterols, triterpene alcohols, and phospholipids in seed oil from white lupin. *J. Am. Oil Chem. Soc.,* 79: 1201-5.

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