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Pharmacological Evaluation of Parmelioid Lichen *Flavoparmelia Caperata (L)* Hale WithSpecial Reference To Analgesic and Anti Inflammatory Properties

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Abstract: Lichens depsides play a key role in the pharmacological importance that contributes in managing clinical disorders. This will provide further clues whether the exogenous depsides regulates cell growth, revealing new communication avenues between organisms of different kingdoms. At this juncture, it needs to be stated that such a cross-kingdom communication so far, has not been previously considered for the lichen species of this study. Hence this work may probably, be the first attempt to explore the impact of depsides of parmelioid lichens on animal and microbial kingdom.

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I. INTRODUCTION

Plant products as drugs and herbal remedies have been employed since prehistoric times to treat human and animal diseases and several countries still rely on plants and herbs as main sources of drugs. Plants are known to produce certain bioactive molecules which react with other organism and results in the inhibition of bacterial, fungal and viral growth. Phytochemicals are chemical substances that occur naturally in plants that have protective or disease preventive properties. Each type of plant part such as fruit or vegetable may contain hundreds of photochemical compounds.

Lichens are complex systems of organisms involved in the symbiotic relationship between a cyanobacteria or green alga (phycobiont), or both and with a fungus (mycobiont). Lichens have driven considerable attention because of their apparent place on the hierarchy of evolution to land plants. Lichens are known to produce various secondary metabolites that are distinctive with reverence to those of higher plants. Many of the lichen substances are used in pharmaceutical sectors. Lichen extracts have been used for various remedies in folk medicine. Screening of lichen extracts has made known the recurrent incidence of these metabolites with antimycobacterial, antibiotic, antitumor, antiviral,analgesic and antipyretic properties. Lichen and lichen products have been used in conventional medicines for centuries and still hold considerable interest as alternative treatments in various parts of the world. Their effectiveness is due to the synthesis of inimitable secondary metabolites that have important biological roles.

Lichens are deliberate emergent associations of fungi (known as mycobionts) and its photosynthetic partners (photobionts) could be of blue green algae of Cyanobacteria. The entire thallus part of lichens are susceptible to infiltration and accrual of airborne elements, they are essential for proper functioning of the lichen but others are toxic. These descriptions pooled with their capability to grow in a wide range of ecological area ranks them among the best bio-indicators of air pollution. Amalgamation and accumulation of airborne elements by lichens, including heavy metals, and their associated physiological responses have been studied widely and utilized to aid in construalof epidemiological studies on human respiratory disorders.

II. MATERIALS AND METHODS

Collection and identification of lichen

Lichens species were collected from high altitudes of Eastern (Kolli hills) Ghats of Tamilnadu state. The collected lichen sample was systematically identified at Botanical Survey of India, Allahabad. Lichen species were ground and added to 500 ml of different solvents, Ethyl acetate extract (EAE) and Diethyl ether extract (DEE).

The phytochemical analysis of the lichen extracts compounds was qualitatively analyzed as described by Harborne (2005).

Bacterial and fungal cultures were identified in these studies based on their pathogenicity, and were obtained from MTCC, IMTECH - Chandigarh. Bacterial strains tested were *Escherichia coli* (MTCC-1650), *Staphylococcus aureus* (MTCC-3160), *Klebsiella pneumoniae* (MTCC-7028), *Pseudomonas fluorescens* (MTCC-2268), and *Bacillus subtilis* (MTCC-3053). Fungal strains selected were *Pestalotia foedans* (MTCC-934), *Phomopsis leptostromiformis var. occidentalis* (MTCC-2382) (grown in Oatmeal agar), *Fusarium*

oxysporum (MTCC-6338) (grown on potato dextrose agar), *Paeciliomyces variotii* (MTCC-1368) (grown in Czapek's agar). Antibacterial and antifungal activity of lichen species by agar well diffusion method.

Evaluation of anti oxidant properties of lichen extracts

Catalase activity of the solvent extracts of *Flavoparmelia caperata* were determined. The activity of peroxidase in solvent extracts of *Flavoparmelia caperata* was estimated according to the method described by Reddy *et al*., (1995). The Glutathione-S-transferase activity was estimated by conjugating GSH with chloro - 2, 4 dinitrobenzene (CDNB) and the extent of conjugation is used as a measure of enzyme activity from the proportionate change in the absorption at 340 nm (Habig *et al*., 1974). Reduced glutathione levels were estimated by the method of Moron *et al*., (1979). The ability of lichen extracts to scavenge the stable free radical DPPH was determined by the method described by Mensor *et al*., (2001). The amount of ascorbic acid present in lichens was estimated according to the method described by Roe and Keuther, (1953). The levels of tocopherol in lichen samples were determined by spectrophotometric method (Rosenberg, 1992).

Acute oral toxicological studies

The acute oral toxicity study was carried out as per the OECD-423 guidelines (Ecobichon, 1997), acute toxicity studies were performed on albino mice of either sex. All the animals used in this study were purchased after proper clearance from the Institutional Animals Ethics Committee organized by the Swamy Vivekanandha College of Pharmacy having approval number: SVCP/IAEC/PG/1/03/2018.

Study of analgesic activity of lichen extracts

Swiss albino miceweighing 25-30 g bred in M/s. Venkateshwara Animal Breeders Pvt. Ltd, Bangalore, was used. The animals were fed with standard food pellets and water *ad libitum*. They were maintained in standard laboratory conditions (12 to 12 hour light and dark cycles with 25 ± 2°C). 36 animals were divided into 6 Groups of 6 animals each and they were fasted overnight during the experiment free access to water.

Standardization of analgesic model I (Hot plate method)

The analgesic activity was evaluated by the method given by Eddy and Leimback (1953), maintained at a temperature of 55 ± 1°C. This method was specifically used to evaluate central analgesic action. Each mice was placed on the hot plate maintained at 55± 1°C, and the response time was recorded as the time at which animals reacted to pain stimulus by either paw licking response or jumping response, whichever appeared first at 30, 60, 90 and 120 min after treatment. The cut off time for the reaction was 15 seconds.Positive groups was treated with tramadol as standard drug. Test groups wre treated with 200 and 400mg intrperitoneally suspended in 1 % CMC of EAE-FC and DEE-FC.

Standardization of analgesic model II(Acetic acid induced writhing method)

The analgesic activity by against acetic acid-induced writhing was evaluated according to the method given by Dey *et al*., 2010. This method was used preferentially to evaluate the peripheral action. The animals were divided into six groups comprising six mice in each group. Thirty minutes after the extract administration, 0.1 ml of 0.6% acetic acid was injected for the induction of writhings in mice. The writhing effect was indicated by stretching of at least one hind limb. This response was observed for 30 min after the acetic acid administration, and reduction in number of writhings in the treated groups and standard were compared with animals in the control group. Writhing was induced with 0.6% v/v acetic acid negative control. Aspirin was given as standard to treat writhing in positive control group. Test groups wre treated along with acetic acid and 200 and 400mg of EAE-FC and DEE-FC.

The percentage protection of abdominal constrictions was calculated by the formula:

Protection (%) = Wc –Wt/ Wc × 100

Wc = Mean no. of writhes (Control),

Wt/Wc = Mean no. of writhes (Test/Standard).

Study of anti-inflammatory activity of lichen extracts

Albino Wistar Rat weighing 180-250 g bred in M/s. Venkateshwara Animal Breeders Pvt. Ltd, Bangalore, was used. The animals were fed with standard food pellets and water *ad libitum*. They were maintained in standard laboratory conditions (12 to 12 hour light and dark cycles with 25 ± 2°C). 36 animals were divided into 6 Groups of 6 animals each and they were fasted overnight during the experiment free access to water.

Standardization of anti-inflammatory model

Anti-inflammatory activity was determined according to Winter *et al.* (1962). Rats were divided into 6 groups of six individuals and food withheld overnight before the experiment. Treatment groups included oral

administration of the lichen extracts. After 30min, inflammation was induced by a single sub-plantar injection of carrageenan (0.1 ml of 1% w/v in normal saline), into the left hind paw of each rat. The paw volume was chemically induced with the oral administration of carrageenan (1% v/v) in negative control group. Positive control group was treated along with carrageenan and Indomethacin (20mg/kg) suspended in CMC. Test group animals (each six individuals) were treated with 200 and 400mg of EAE and DEE of *Flavoparmeliacaperata*.The paw oedema volumes were measured with plethysmometer before and as well as 1 hour interval from 1 to 3hrs after carrageenan exposure. The inhibition rate of oedema was calculated as percentage as follows.

Inhibition % = $\frac{Vc-Vt}{Vc} \times 100$

Where: Vc and Vt are the rate of oedema development/inhibition of control and treated groups, respectively.

III. RESULTS

The species was identified and deposited (Ref No. BSI/CRC/Tech-Authent./2017-18-10499) at Botanical Survey of India, Allahabad.

Phytochemical analysis

The presence or absence of specific phytochemical, found in the lichen extracts was determined and presented in the table: 1.

TEST	EAE-FC	DEE-FC
ALKALOIDES		
Dragandorff's test	**+ve**	**-ve**
Mayer's test	**-ve**	**-ve**
Wagner's test	**+ve**	**+ve**
PHENOLIC COMPOUNDS		
Lead acetate test	**-ve**	**+ve**
FLAVONOIDS		
Shinoda's test	**-ve**	**-ve**
Ferric chloride test	**+ve**	**-ve**
Sodium hydroxide test	**+ve**	**-ve**
GLYCOSIDES		
Killer Kiliyani test	**-ve**	**-ve**
Legal's test	**-ve**	**+ve**
TERPENOIDS		
Chloroform - H_2SO_4 test	**-ve**	**+ve**
TANNINS		
Ferric chloride test	**-ve**	**-ve**
SAPONINS		
Sodium carbonate test	**+ve**	**-ve**

Table: 1- Phytochemical analysis of ethyl acetate and diethyl ether extracts of *Flavoparmelia caperata*. EAE-Ethyl acetate extract; DEE-Diethyl ether extract.

Phytochemical compounds are specific metabolites which act as antioxidants (Oktay *et al*., 2003). They involve in oxidation-reduction systems allowing them to act as the donors of electron, hydrogen and also quench singlet oxygen molecules. Lichens are symbiotic associations of fungi and algae which produce numerous secondary metabolites, such as amino acids, sugar alcohols, aliphatic acids, macrocyclic lactones, monocyclic aromatic compounds, quinines, chromones, xanthones, dibenzofurans, depsides, depsidones, depsones, terpenoids, steroids, carotenoids and diphenyl ethers (Kahkonen *et al*., 1999). Phenolic compounds are one of the largest and ubiquitous groups of plant secondary metabolites (Singh *et al*., 2007). They acquire numerous activities such as anti-apoptotic, antiaging, anticarcinogenic, anti-inflammatory, anti-arthrosclerotic, and cardiovascular protective and improves endothelial function as well as inhibition of angiogenesis and cell proliferation activities (Han *et al*., 2007). Several studies have been described the antioxidant properties of medicinal plants that are rich in phenolic compounds (Brown and Rice-Evans, 1998).

Antimicrobial activity

The EAE of *F.caperata* has shown varying degree of effectiveness against the tested bacterial and fungal species. Among the bacterial species tested, *Pseudomonas fluorescens* have shown highest sensitivity (15mm), (P<0.001) which is highly significant followed by *Klebsiella pneumonia* (15mm), (P>0.05) which is non significant and moderate sensitivity by *Escherichia coli* and *Staphylococcus aureus* (14 mm), (P>0.05) which is non significant.

BACTERIAL/FUNGAL SPECIES	EAE	DEE	S/K
Escherichia coli	**14±1.7^{NS}**	**16±0.35^{NS}**	**17±1.2**
Staphylococcus aureus	**14±0.26^{NS}**	**21±0.55*** **	**25±0.70**
Klebsiellapneumonia	**15±1.45^{NS}**	**20±0.57** **	**20±0.85**
Pseudomonas fluorescens	**15±0.49*** **	**14±0.52*** **	**11±0.70**
Bacillus subtilis	**12±0.40^{NS}**	**14±0.61** **	**18±0.94**
Pestalotiafoedans	**17±0.90^{NS}**	**11±0.20*** **	**15±0.95**
Phomopsisleptostromiformis var. occidentalis	**13±0.62** **	**16±0.61** **	**10±0.34**
Fusariumoxysporum	**19±1.36*** **	**15±0.43*** **	**15±1.35**
Paeciliomycesvariotii	**15±1.45^{NS}**	**13±0.70** **	**12±0.40**

Table: 2 - Antimicrobial activity of *Flavoparmeliacaperata*. Values (Zone of inhibition in mm) are expressed as mean ± SEM (n = 6). *** - *P*< 0.001, ** - *P*< 0.01, * - *P*< 0.05, NS - non significant (*P*> 0.05). EAE-Ethyl acetate extract; DEE-Diethyl ether extract.[S- Streptomycin: Standard drug for bacterial strains; K-Ketaconazole: Standard drug for fungal strains].

Among the fungal species, highest effect was shown against *Fusarium oxysporum* (19mm)(P<0.001) that have shown highly significant effectiveness, followed by *Pestalotia foedans* (17mm) (P>0.05) which have non significant effectiveness. Least sensitivity was shown by *Phomopsis leptostromiformis var. occidentalis* (13 mm) (P<0.01) that revealed moderate significant effectiveness (Table-2).

The DEE of *F. cooperation*had also shown different pattern effectiveness against the tested bacterial and fungal species. Among the bacterial species tested, greatest effectiveness was shown against *Staphylococcus aureus* Among the tested fungal species, *Phomopsis leptostromiformis var. occidentalis* (16mm) (P<0.01) with moderate significance, have shown greatest sensitivity, followed by *Fusarium oxysporum* (15mm) (P<0.001) with greater significance, and *Paeciliomyces variotii* (13 mm) (P<0.01) exhibited moderate significance. Greater resistance was shown by means of least sensitivity by *Pestalotia foedans* (11 mm) (P<0.001) having greater significance (Table-2).

From our results, it was revealed that Gram positive bacteria were more sensitive to DEE of *F.caperata*, which may be due to the presence of simple outer membrane that induces the degree of membrane permeability of cell walls, but in case of the Gram negative bacterial cell wall is made up of thick peptidoglycan, lipopolysaccharides, Lipo and glyco proteins contributing to0 the poor permeability of the lichen secondary metabolites (Vattem *et al*., 2004). Greater the resistance of Gram negative bacteria to the lichen extracts in this study indicates that the murine membrane is the principle layer found in the outer cell membrane prevents the incoming of active molecules inside the cell and the lipopolysaccharide coat on the outer part, followed beneath by a slender layer of peptidoglycan (Palombo and Semple., 2001). The presence of relatively high content of apolar lichen compounds interrupting the permeability bacterial cell wall (Tay *et al*., 2004).

Among all the seven tested extracts, diethyl ether extract was found to be more effective than other extracts, which is released as lichen derived ester compounds impede with the ATP synthase inhibitory activity on the cell wall (Gardiner *et al*., 2005). Scabrosin, a lichen derived ester was previously reported for their ATP synthase inhibitoryactivity, thereby dictates the influence of ATP production in mitochondria. When the mitochondrial ATP synthase is inhibited, the membrane is hyperpolarized and leads to apoptosis (Gardiner *et al*., 2005).

The process of acidification of plasma membrane surface of a microorganism, resulting in proton - ATPase release leads to break that is required for ATP synthesis (Vattem *et al*., 2004; Maeda *et al*., 1999). In addition, it also causes intracellular coagulation of cytoplasmic organelles, leads to cell death, or growth inhibition (Adham *et al*., 1998). The antibacterial effect is relatively stronger than antifungal effect. This observation coincides with other studies (Branislav *et al*., 2011). The above results obtained due to the fact that the difference in sensitivity between fungi and bacteria can be found due to the transparency of the cell wall (Yang and Anderson, 1999). In this work, antimicrobial activity was evaluated in terms of antibacterial and antifungal activities of ethyl acetate and diethyl ether extracts of *F.caperata*. The data presented in this study have significant antimycobacterial influence relative to the tested bacteria and fungi. For certain plant diseases caused by fungus, these above mentioned bioactive extracts of different lichen species can be formulated for improving the quality of bio fungicides which at both ways kills plant insect pests as well as fungal pathogens.

Antioxidant activities of lichen extracts

The levels of ascorbic acid were found to be higher in EAE significantly compared with DEE of *F.caperata*. The levels of tocopherolwere moderately higher in ethyl acetate (P<0.01) compared with ether extract. In case of the levels of peroxidase, it was higher in ether extract (P<0.001) which is significantly higher than ethyl acetate extract. The levels of catalase were found to be moderately higher (P<0.01) in DEE when compared to EAE of *F.caperata* (Table-3).

ANTIOXIDANT	EAE	DEE
Ascorbic acid (mg/gram fresh extract)	24.29±0.33***	12.7±0.09
Tocopherol (mg/gram fresh extract)	1316.84±30.16**	1161.98±8.02
Catalase (Units/gram fresh extract)	67.48±1.43	103.01±1.43**
Peroxidase (Units/gram fresh extract)	122.4±1.65	842.12±6.62***
Glutathione - S - Transferase(Units/gram fresh extract)	412.41±17.75*	343.64±34.25
Reduced glutathione (nano moles/gram fresh extract)	5.63±0.32	6.25 ± 3.24^{NS}
DPPH scavenging activity (%/gram fresh extract)	64.36±1.37**	46.28±0.74

Table: 3 - Antioxidant properties of ethyl acetate and diethyl ether extracts of *Flavoparmeliacaperata*. The levels are expressed as mean ± SEM (n = 6). *** - *P*< 0.001, ** - *P*< 0.01, * - *P*< 0.05, NS - non significant (*P*> 0.05). EAE-Ethyl acetate extract; DEE-Diethyl ether extract.

Decreased catalase activity in EAE of *F.caperata* might be due to the translation of catalase into its inactive products by associating with H_2O_2 (Schonbaum and Chance, 1976). *Flavoparmalia caperata*was found to grow in high moisture areas like tree barks. Trees that are lithotrophic, mainly depend on rocky silicas. Such lichen thallus frequently exposed to the huge moisture content. The rhizinomorphs are highly thin and elusive to flooding and moisture accretion. In order to sheltered themselves from obliteration by moisture, they possess a set of enzymes that could degrade oxidized water into water. Since catalase plays a vital role in lichens having an impact on the environment.

The activity of peroxidase shown to rise in overwintering organs, while the enzyme activity decreased with spring and summer. The GSH levels were comparatively higher in DEE of *F. caperata*. Glutathione found in its reduced state (GSH) in *L. polytropa* apothecia, suggesting that there will no serious oxidative stress events in copper rich lichen. Desperately to higher plants, GSH may be the main non enzymatic antioxidant in lichens (Kranner *et al*., 2005). Glutathione, the chief low molecular thiol substance present in living systems, playing a crucial role in exhibiting cellular defence mehanism against oxidative damage consequences. The GSH level in the copperized apothecia of *L. polytropa* was two to four times lesser than those in foliose lichens such as *P. furfuracea, L. pulmonaria, Peltigera polydactylon* and *Xanthoria parietina* (Kranner, 2002; Pawlik *et al*., 2002) that generally are not exposed to metals. (Pawlik, 2002).

The DPPH scavenging activity of EAE of *F.caperata* is comparatively higher than that of DEE. The radical properties of DPPH were lost because of the donation of hydrogen ion by lichen extract leads to the formation of a stable protonated DPPH molecule (Confort*iet al*., 2008). Lichen compounds with high phenolic

content have shown stronger scavenging of DPPH radicals. The results obtained are similar to previous studies where extracts containing high phenolic contents exhibited stronger scavenging activities (Poornima *et al*., 2012). The EAE of *F.caperata* have comprised high phenolic content have shown maximum DPPH activity (64.36%) when compared to DEE. Preceding investigation on the antioxidant potential of *Parmotrema pseudotinctorum* (Kumar *et al*., 2010) and *Everniastrum cirrhatum* (Kekuda *et al*., 2011) have shown analogous results in which the extract was found to display lesser potential of free radical scavenging capacity when compared to standard reference.

Acute toxicity studies

Both EAE and DEE of *F.caperata* have produced no toxic symptoms up to a dose level of 2000 mg/kg body weight orally inrats, and hence the extracts were considered safe for furtherpharmacological evaluation methods. Hence 1/10th and 1/5th(200mg and 400mg respectively) of solvent extracts were considered for all *in vivo* experiments as effective doses.

Analgesic activities of lichen extracts by hot plate method

The analgesic activities (hot plate method) of EAE and DEE of *F.caperata* were presented in the table 4. In hot plate method, a significant increase in the retention time was found in group treated with 400mg of EAE, and in groups treated with 200 and 400 mg of DEE at 90 minutes. A moderate significance (P<0.01) was found in group treated with 400 mg of EAE and 200 mg of DEE at 60 minutes. A highly significant increase (P<0.001) was found in groups treated with 400 mg of EAE, and 200 and 400 mg of DEE treated group at 120 minutes. Among the tested group, a very significant (P<0.001) increase in retention time was observed in 90 minutes in the group treated with 200 mg of DEE. But such increase in RT was observed in 60 minutes itself (12.30 ± 0.85) (P<0.001) in the group treated with DEE at 400 mg.The hot plate method is the general procedure to screen the centrally acting analgesic compounds. It scales the complex response to a non-inflammatory and acute nociceptive that are normally used for studying the nociceptive activity (Sabina *et al*., 2009). It is a well established piece of evidence that any agent that causes a persistence of the hot plate latency by this model must be acting centrally (Ibironke and Ajiboye, 2007). This study dictated that that *F.caperata* possessed low to moderate analgesic activity in a dose dependent manner on Eddy's hot plate method.

Group	Dose	Reaction time in Sec. (Mean ± SEM)				
		Basal	30 min	60 min	90 min	120 min
Control	-	6.50 ± 0.64	7.00 ± 0.40	7.50 ± 0.64	8.00 ± 0.38	7.00 ± 0.41
Tramadol	10 mg/kg	6.25 ± 0.48	9.00 ± 0.63*	11.50 ± 0.96***	13.00 ± 0.40***	11.00 ± 0.48***
Ethyl acetate	200 mg/ kg	6.75 ± 0.63	7.75 ± 0.85	10.00 ± 0.82	9.80 ± 0.63**	10.00 ± 0.4***
Ethyl acetate	400 mg/kg	6.50 ± 0.29	7.75 ± 0.91	11.50 ± 0.65**	12.50 ± 0.29***	11.00 ± 0.91***
Diethyl ether	200 mg/kg	6.25 ± 0.48	8.00 ± 0.40	11.30 ± 1.10**	14.80 ± 0.71***	10.75 ± 0.85***
Diethyl ether	400 mg/kg	6.50 ± 0.65	7.50 ± 0.65	12.30 ± 0.85***	13.00 ± 0.71***	11.25 ± 0.41***

Table: 4 - Analgesic activity (hot plate method) of ethyl acetate and diethyl ether extracts of *Flavoparmeliacaperata*. The levels are expressed as mean ± SEM (n = 6). *** - *P*< 0.001, ** - *P*< 0.01, * - *P*< 0.05.

Analgesic activities of lichen extracts by acetic acid-induced writhing in mice

The analgesic activities (acetic acid-induced writhing) of EAE & DEE of *F.caperata* were presented in the table 5. A moderate significant increase (P<0.01) in the number of writhings were observed in the all the test groups (200 and 400 mg of EAE and DEE treated groups) when compared with the control group. The percentage of protection was found to be not having much significant change as and when compared with aspirin treated group. But a closely related percentage of protection was observed in the group treated with 400 mg of EAE (39.3%).

The effect of EAE and DEE against the noxious stimulus may be a depression in the production of irritants and thereby reduction in the number of writhings in mice. The abdominal muscle contraction is very

sensitive induced by acetic acid to explore peripherally acting antinociceptives. This response is believed to involve local peritoneal receptor expression (Chakraborty et al., 2004). The result of the current study indicates that the analgesic effect of *F.caperata* might be mediated by inhibition of the synthesis of receptors mediators there establishing the action of the extracts as peripherally acting nociceptives.

Group	Dose	No. of writhing (in 10 min)	Protection (%)
Control	-	10.5 ± 0.96	-
Aspirin	5 mg/kg	5.5 ± 0.65***	47.6%
Ethyl acetate	200 mg/ kg	7.5 ± 0.50**	28.6%
Ethyl acetate	400 mg/kg	7.0 ± 0.58**	39.3%
Diethyl ether	200 mg/kg	7.5 ± 0.96**	28.6%
Diethyl ether	400 mg/kg	7.5 ± 0.50**	28.6%

Table: 5 - Analgesic activity (acetic acid-induced writhing) of ethyl acetate and diethyl ether extracts of *Flavoparmeliacaperata*. The levels are expressed as mean ± SEM (n = 6). *** - $P< 0.001$, ** - $P< 0.01$

From the results, it was confirmed that in hot plate method, the analgesic activity of EAE (group treatment at 400 mg) of *F.caperata* was found to be more or less equivalently, when compared to standard drug aspirin, whereas, the diethyl ether extract have shown a lesser effect than the standard drug treated animals

Anti-inflammatory effect of lichen extracts

The anti-inflammatory effect of the lichen extracts is presented in the table 6. The paw oedema volumes have shown varying degree of changes in treated and induced groups. After 1 hour of treatment, none of the group has shown any changes in paw volume. But when time prolongs the induced paw had shown remarkable changes in terms of reduction of paw volume. It was observed that the treated group (200 mg DEE) has shown moderate significance (P<0.01) decrease in paw volume in 2 hours and significant decrease when time extends to 3 hours having very significant decrease (22.1%; P<0.001) of paw volume. Remaining groups treated with higher dosages of EAE and diethyl ether extracts doesn't show any significant changes in the reduction of paw volume.(Table-6).

Group	Dose	% inhibition of paw volume			
		0 hrs	1 hr	2 hrs	3 hrs
Control	-	0.43 ± 0.05	0.50 ± 0.04	0.65 ± 0.05	0.68 ± 0.03
Indomethacin	2mg/kg	0.36 ± 0.03	0.40 ± 0.01***(20 %)	0.58 ± 0.04**(36.9%)	0.55 ± 0.05(45.6%)
Ethyl acetate	200 mg/ kg	0.35 ± 0.03	0.48 ± 0.03 (4 %)	0.56 ± 0.09 (10.8%)	0.55 ± 0.10(19.1%)
Ethyl acetate	400 mg/kg	0.36 ± 0.03	0.48 ± 0.05 (4 %)	0.62 ± 0.04* (23 %)	0.53 ± 0.08(22.1%)
Diethyl ether	200 mg/kg	0.36 ± 0.05	0.43 ± 0.05*(14 %)	0.41 ± 0.03**(18.5%)	0.37±0.02***(22.1%)
Diethyl ether	400 mg/kg	0.43 ±0.05	0.45 ± 0.03 (10 %)	0.50 ± 0.04*(23 %)	0.48±0.05*(29.4%)

Table: 6 - Anti inflammatory effect of ethyl acetate and diethyl ether extracts of *Flavoparmeliacaperata*. The values are presented as mean ± standard error mean (n = 6). Star denotes the significance levels in comparison with the negative control: $*P< 0.05$; $**P< 0.01$; $***P< 0.001$.

The progression and establishment of carrageenan induced oedema comprises two phases; the first phase is ascribed to the release of histamine, serotonin and kinins (which are generally primary inflammatory mediators). In the late second phase various sterol based derived lipids such as prostaglandins and secondary inflammatory mediators that includes bradykinins were released contributing to the entire inflammatory process to be executed that canbe measured after 3 hours of induction (Brooks and Day, 1991; Vane and Botting, 1987).

IV. CONCLUSION

The anti-microbial property of the DEE and EAE extracts of *F.caperata* were explored. Subsequently, its antioxidant, acute toxicity studies, analgesic and anti-inflammatory activities were investigated. The above study has produced notable declarations that, these extracts may be used identifying, analyzing, and purifying the lead molecule for exploring the frontiers of *F. caperata*. The crude extracts now can be conveniently recognized in validating their efficacy in relative disorders in laboratory animals, to cross over varying degrees of preclinical and clinical trial phases, henceforth, one come out with new and modified lead molecules that help the human society in preventing from diseases and disorders.

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REFERENCES

[1]. Adham IAJ, Dinning I, Eastwood P, Austin, Collie PJ. Cell membrane effects of some common biocides. J Ind Microbiol and Biotech. 1998; 21 : 6.
[2]. Branislav R Rankovic, Marijana M Kosanic, Tatjana P Stanojkovic. Antioxidant, antimicrobial and anticancer activity of the lichens *Cladoniafurcata*, *Lecanoraatra* and *Lecanoramuralis*. BMC Comp Alt Med. 2011; 11: 97 - 104.
[3]. Brooks PM, Day RO. Nonsteroidal antiinflammatory drugs--Difference and similarities. N Engl J Med. 1991;324(24):1716-1725.
[4]. Brown JE, Rice-Evans CA. Luteolin rich artichoke extract protects low density lipoprotein from oxidation in vitro. Free Radical Res. 1998; 29: 247 - 255.
[5]. Chakraborty AR, Devi RK, Rita S, Sharatchandra KH, Singh TI. Preliminarystudies on antiinflammatory and analgesic activities of *Spilanthesacmella* in experimental animal models. Indian J Pharmacol. 2004; 36 (3): 148-150.
[6]. Dey YN, De S, Ghosh AK. Evaluation of analgesic activity of methanolic extract of Amorphophallus paeonifolius tuber by tail flick and acetic acid induced writhing response method. Int J Pharm Bio Sci. 2010; 1 (4): 662-668.
[7]. Ecobichon DJ. The basis of toxicity testing.CRC Press, New York; 1997.
[8]. Eddy NB, Leimback D. Synthetic analgesics II. Dithienylbutenyl and dithienyl butylamines. J Pharmacol Exp Ther 1953; 107: 385-393.
[9]. Gardiner DM, Warning P, Howlett BJ. The epipolythiodioxopiperazine (ETP) class of fungal toxins: Distribution, mode of action, functioning and biosynthesis. Microbiology. 2005; 151: 1021 - 1032.
[10]. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974; 246: 7130 - 7139.
[11]. Han X, Shen T, Lou H. Dietary polyphenols and their biological significance. Int J Mol Sci. 2007; 950 - 988.
[12]. Ibironke GF, Ajiboye KI. Studies on the anti-inflammatory and analgesic properties of *Chenopodiumambrosioides* leaf extract in rats. Int J Pharm. 2007; 3 (1): 111-115.
[13]. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS. Antioxidant activity of plant extracts containing phenolic compounds. J Agri Food Chem.1999; 47: 3954 - 3962.
[14]. Kekuda TRP, Vinayaka KS, Swathi D, Suchitha Y, Venugopal TM, Mallikarjun N. Mineral composition, total phenol content and antioxidant activity of a macrolichen *Everniastrumcirrhatum* (Fr). Hale (*Parmeliaceae*). E-Journal of Chemistry. 2011; 8 (4): 1886 – 1894.
[15]. Kranner I, Cram WJ, Zorn M, Wornik S, Yoshimura I, Stabentheiner E, Pfeifhofer HW. Antioxidants and photo protection in lichen as compared with its isolated symbiotic partners. Proc Nat Aca Sci. 2005; 102: 3141 - 3146.
[16]. Kranner I. Glutathione status correlates with different degrees of desiccation tolerance in three lichens. New Phytologist. 2002; 154: 451 - 460.
[17]. Kumar PSV, Kekuda PTR, Vinayaka KS, Sudharshan SJ, Mallikarjun N, Swathi D. Studies on antibacterial, anthelmintic and antioxidant activities of a macrolichen *Parmotremapseudotinctorum* (des.

Abb.) Hale (Parmeliaceae) from Bhadra wildlife sanctuary, Karnataka. Int J PharmTech Res. 2010; 2 (2): 1207 - 1214.

[18]. Mensor LL, Menezes FS, Leitao GG, Reis AS, Santos TC Coube CS, Leitao SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res. 2001; 15: 127 – 130.

[19]. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glulathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochem Biophys Acta. 1979; 582: 67 - 78.

[20]. Oktay M, Gulcin I, Kufrevioglu OI. Determination of in vitro antioxidant activity of fennel (*Foeniculumvulgare*) seed extracts. LWT. 2003; 36: 263 - 271.

[21]. Palombo EA, Semple SJ. Antibacterial activity of traditional medicinal plants. J Ethnopharmacol. 2001; 77: 151 - 157.

[22]. Pawlik-Skowronska B, Sanita di Toppi L, Favali MA, Fossati F, Pirszel J, Skowronski T. Lichens respond to heavy metals by phytochelatin synthesis. New Phytologist. 2002; 156: 95 - 102.

[23]. Pawlik-Skowronska B. Correlations between toxic lead effects and production of lead induced thiol peptides in the microalga *Stichococcusbacillaris*. Environmental Pollution. 2002; 119: 119 - 127.

[24]. Poornima G, Kekuda PTR, Vinayaka KS. Antioxidant efficacy of Olea dioica Roxb (Oleaceae). Biomedicine. 2012; 32 (4): 506 - 510.

[25]. Reddy KP, Subhani SM, Khan PA, Kumar KB. Effects of light and benzyl adenine and dark treated growing rice (Oryza sativa) leaves changes in peroxidase activity. Plant Cell Physiol. 1995; 26: 987 - 994.

[26]. Roe JH, Keuther CA. The determination of ascorbic acid in whole blood and urine through 2, 4 - dinitrophenyl hydrazine derivative dehydro ascorbic acid. J Biol Chem. 1953; 147: 399 - 407.

[27]. Rosenberg HR. In: Chemistry and Physiology of Vitamins. Inter science Publishers; New York, 1992; 452 - 453.

[28]. Sabina E, Chandel S, Rasool MK. Evaluation of analgesic, antipyretic and ulcerogenic effect of Withaferin A. Int J Integ Bio. 2009;6(2):52-56.

[29]. Schonbaum GR, Chance B. Catalase. In: The enzymes, 2nd Edn, Boyer PD (Ed). Academic press; New York, 1976; 13: 363 - 408.

[30]. Singh R, Singh SK, Arora S. Evaluation of antioxidant potential of ethyl acetate extract/fractions of Acacia auriculiformis. 2007; 45: 1216 - 1223.

[31]. Tay T, Ozdemir TA, Yilmaz M, Turk H, Kivanc M. Evaluation of the antimicrobial activity of the acetone extract of the lichen *Ramalinafarinacea* and its (+) - usnic acid, norstictic acid and protocetraric acid Constituents. Z Naturforsch C. 2004; 59: 384 - 388.

[32]. Vane J, Botting R. Inflammation and the mechanism of action of anti-inflammatory drugs. FASEB J. 1987; 1 (2): 89-96.

[33]. Vattem DA, Lin YT, Lable RG, Shetty K. Phenolic antioxidant mobilization in cranberry pomace by solid-state bioprocessing using food fungus Lentinus edodes and effect on antimicrobial activity select food borne pathogens. Innov Food Sci Emerg Tech. 2004; 5: 81 - 91.

[34]. Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Carrageenan-induced edemas in hind paw of the rats as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. 111, 544-547.

[35]. Yang Y, Anderson EJ. Antimicrobial activity of a porcine myeloperoxidase against plant pathogenic bacteria and fungi. J App Microbiol. 1999; 86: 211 - 220.