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Anti-inflammatory, Analgesic, Anti-pyretic, Anti-ulcer and CNS Stimulant Activities of Marine Fungi *Aspergillus oryzae*

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ABSTRACT

Objective: To assess the anti-inflammatory, antiulcer, antipyretic, analgesic and CNS stimulant activities of crude extract of marine fungi *Aspergillus oryzae*. **Methods:** The fungi *Aspergillus oryzae* was isolated from mangrove *Avicennia marina* rhizosphere sediment and cultured in Glucose Peptone Yeast extract broth. The culture supernatant was extracted with equal volume of ethyl acetate solvent and concentrated through evaporation. The extract was investigated for pharmacological properties by following standard methods using adult albino rats. **Results:** In the anti-inflammatory assay, extract 100 mg kg^{-1} showed significant activity. The analgesic activity of the extract at 200 mg kg^{-1} was equal to that of positive control (Dichlofenac sodium) and was showed highly significant ($p<0.001$) when compared to the negative control. The extract at 200 mg kg^{-1} showed dose dependent and significant ($p<0.5$) antipyretic activity. Test extract at 100 mg kg^{-1} showed substantial stimulant activity (19.5%) comparable to that of positive control (caffeine). The fungal extract at 200 mg kg^{-1} significantly reduced ($p<0.001$) the volume of gastric juice, free and total acidity, alkaline phosphatase and increase calcium level indicating the antiulcer activity against gastric pyloric ulcers. **Conclusion:** The study indicated that the fungi *A. oryzae* extract possess pharmacological properties.

Key words: *Aspergillus oryzae*, marine fungi, anti-inflammatory, antiulcer, analgesic, antipyretic, CNS stimulant

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INTRODUCTION

Most of the pharmaceutical compounds are not effective in treatment of human diseases due to the growing incidence of drug-resistance. In this scenario, marine fungi derived secondary metabolites are structurally unique and possess interesting biological and pharmacological properties. The productions of these compounds mainly depend on the nutrition, temperature variations, high pressures, salinity and competition with other microbes. They may have developed some specific secondary metabolic pathway for production of bioactive metabolites[1]. Thus, marine fungi offer a wonderful resource for the discovery of new compounds and compounds with new activity.

Usually pharmacological properties of any drug is assessed through various common pharmacological tests such as anticoagulant, analgesic activity, anthelmintic activity, antipyretic activity, antiallergic activity, antiarhythmic activity, immunomodulatory activity, central nervous system activity, antiulcer activity, toxicity assay and antidiabetic activity[2,3].

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Inflammation is typically a protective mechanism that is triggered by inflammatory mediators and controlled by proinflammatory cytokines. Overproduction of this leads to pathogenesis of various diseases. The untreated inflammation leads to chronic inflammatory disorders[4,5,6]. In general, non steroid drugs were being used for the treatment of inflammation[7] and found not to be very effective against inflammation. Nowadays, steroids and cyclooxygenase inhibitors are commonly being used as anti-inflammatory drugs. But they are causing side effects such as gastro intestinal irritation, ulcers, hypertension and cardiac abnormalities[8]. Hence, there is greater interest to search more compounds of anti-inflammation properties without side effects.

Recently, many new compounds have been derived from marine-derived fungi. Fungal genera *Aspergillus* has been known to be a major contributor to the secondary metabolites of marine fungal origin[9,10,11,12]. Among them Oxygenated Hexylitaconates from a marine sponge-derived fungus *Penicillium* sp. exhibited potent anti-inflammatory activity[13]. So, the present study was aimed at to study the pharmacological potential especially the anti-inflammatory, analgesic, antipyretic, Central Nervous System (CNS) stimulant and anti ulcer

activities of the crude ethylacetate extract of the marine fungi *Aspergillus oryzae.*

MATERIALS AND METHODS

Extraction: *Aspergillus oryzae* (JN86006) was isolated from mangrove *Avicennia marina* rhizosphere sediment and cultured in 100 mL Glucose Peptone Yeast extract broth for seven days at 120 rpm at room temperature. The culture broth was centrifuged at 5000 rpm for 15 min and then supernatant was extracted employing liquid-liquid extraction[9]. Equal volume of ethyl acetate solvent was added to culture broth and solvent phase was separated in a separating funnel and then concentrated by evaporation and used for the assessment of pharmacological activities through pharmacological assays.

Experimental animals: All the experiments were performed with the approval of the protocol by the Institutional Animal Ethics Committee (IAEC). Approximately, 100-200 g of adult albino rats[14] were maintained in a room at a controlled temperature of 22±2°C for 12-h light/dark cycle and relative humidity of 60±5% in Department of Pharmacology, S.B. College of Pharmacy, Sivakasi and were given uniform pelleted diet and water *ad libitum.*

Anti-inflammatory activity: Carrageenan induced paw edema was done by following the method of Winter *et al.*[15]. Rats were divided into four groups of 4 animals each. Edema was induced in sub plantar region of right hind paw of rats by an injection of lamda carrageenin (sigma, 0.05 mL of 1% w/v in saline solution). Saline (1 mL kg^{-1}) given to Group I was used as control and standard drug Diclofenac sodium (10 mg kg^{-1}) was administered to Group II rats and considered as positive control. One hour prior to carrageenan injection, Group III and IV were treated with test extract of *A. oryzae* (in 0.5% gum acacia) at a dose level of 100 and 200 mg kg^{-1} p.o. All the doses were administered orally. The paw volume of each animal was estimated by mercury displacement method using plethysmometer before and after injection at a time intervals of 0, 1, 2, 3, 4 and 5 h.

Analgesic activity: Tail immersion method was done to determine the analgesic activity by following the method of Hukkeri *et al.*[16] and Jain *et al.*[17]. Adult albino rats were tested for their sensitivity by placing the tip of tail (last 1-2 cm) gently in warm water maintained at 55±2°C. Any rats flicking the tail within 5 sec were selected for this experiment. The selected rats were divided into four groups of four animals each and fasted overnight but during the experiment given water only. Group I (control group) received normal saline, Group II (standard reference group) was treated with Dichlofenac sodium (100 mg kg^{-1}) p.o. Group III and Group IV received ethyl acetate extracts of *A. oryzae* at 100 mg and 200 mg kg^{-1} p.o. respectively. After drug treatment, the basal reaction time of all groups of rats was noted at different time intervals of 0, 1, 2 and 3 h.

Antipyretic activity (Yeast induced pyrexia method): Antipyretic activity was performed by following the method of Hukkeri *et al.*[16] and Jain *et al.*[17]. The animals with rectal temperatures of 37.5±0.5°C were selected and divided into four groups each containing six rats. A suspension of Brewer's yeast (15%) in saline (0.9%) was prepared. Fever was induced by the injection of brewer's yeast suspension (10 mg kg^{-1}) subcutaneously in back below the nape of neck. The injected area was massaged in order to spread the suspension beneath the skin. After 18 h the animals develop 0.5°C rise in the rectal temperature were selected for further studies. Group I (control group) was given normal saline and Group II (standard reference group) was treated with Paracetamol (45 mg kg^{-1}) p.o. Group III and Group IV were treated with *A. oryzae* extract at 100 and 200 mg kg^{-1} p.o., respectively. The rectal temperature was recorded using Digital Telethermometer (TNCO) after 1, 2, 3 and 4 h in all groups.

Central Nervous System (CNS) stimulant activity: Locomotor activity was done by following the method of Ramasamy and Kumar[18]. The computerized locomotion detection system (actophotometer) equipped with photosenser was used to measure spontaneous locomotor activity and rearing. In this experiment, the rat was individually placed in a transparent cage (25×48×18 cm^3) before the administration of vehicle (1% saline) or test extracts and locomotor activity was recorded for 10 min. The animals were divided into four groups to assess the effectiveness of test compounds. Group I was served as an untreated control, Group II was treated with standard caffeine (30 mg kg^{-1}, i.p), Group III and IV were treated with test extract at 100 and 200 mg kg^{-1} of *A. oryzae.* The locomotor activity was observed after 30 min of extract administration for 10 min and the percentage change in activity was calculated by following formula:

$$\text{Percentage (\%)} = \frac{\text{(A-B)}}{\text{A}} \times 100$$

where, A: Before drug treatment, B: After drug treatment.

Anti ulcer activity: Albino rats were divided into four groups of 4 animals each. Animals were fasted for 24 h before the study, but had free access to water. The Group I (control group) was given normal saline and Group II (standard reference group) received Ranitidine at

13.5 mg kg^{-1} p.o. Group III and Group IV were treated with *A. oryzae* extract at 100 and 200 mg kg^{-1} p.o. respectively. The animals were anaesthetized with the help of anesthetic ether; the abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated according to the method of Shay *et al.*[19] and avoids traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. After the animals recovered from anesthesia, test extracts were given orally using gavages. The animals were deprived of food and water post operatively and then sacrificed after 19 h of pyloric ligation.

Blood samples were collected from marginal tail vein of rats and subjected for estimation of serum alkaline phosphatase by using the method of Kind and King[20]. Serum calcium was estimated by ortho cresolphthelin complexone method (OCPC)[21,22]. The abdomen was opened and examined for lesion to determine ulceration score[23]. The cardiac end of stomach was dissected out and the contents were drained into a glass tube. The volume of the gastric juice was measured and centrifuged at 3000 rpm for 10 min. From the supernatant, aliquots (1 mL of each) were taken for the determination of pH, total and free acidity. The results were compared with control.

Statistical analysis: The values were expressed as Mean±S.E.M of 4 animals in each group. The data were then analyzed by one way ANOVA followed by Dunnett's test.

RESULTS

Anti-inflammatory activity: The highest volume of increased paw edema was noticed at 2nd h from injection of carragenan. Test extract showed reduction in paw volume, the activity exhibited at 2nd h was higher than standard drug (Table 1). When compared to the negative control, the positive control and that of extract groups showed significant reduction in paw volume at end of 5th h. Both positive control and that of extracts were highly significant ($p<0.001$) when compared to the negative control.

Analgesic activity: Analgesic activity of ethyl acetate extract of *A. oryzae* is represented in Table 2. The administration of positive control (Dichlofenac sodium) showed higher reaction time during the experiment. The reaction time was increased when increasing extract concentration and time. After one hour the percentage of analgesic activity of extract at 200 mg kg^{-1} was equal to that of positive control, but overall analgesic activity was found to be low. The extract at 100 and 200 mg kg^{-1} and positive control showed highly significant ($p<0.001$) reaction time when compared to the negative control.

Antipyretic activity: Effect of test extract of *A. oryzae* on yeast induced pyrexia in rats is depicted in Table 3. The extract and positive control showed dose and time dependent antipyretic activity. Almost equal effect of reduction in temperature was noticed at a dose of 200 mg kg^{-1} and positive control (Paracetamol). The activity of test extract 200 mg kg^{-1} at 4 h was significant ($p<0.5$) when compared with control. Untreated normal rats did not show any decrease in the body temperature on oral administration of extract.

Central nervous system (CNS) stimulant activity: The locomotor activity was greatly stimulated by test extract and the higher dose showed decrease in stimulant activity (Table 4). The positive control (caffeine) showed maximum stimulant activity (24%). The test extract showed comparatively lower activity than positive control. However, test extract at 100 mg kg^{-1} showed substantial stimulant activity (19.5%).

Table 1: Anti-inflammatory activity of ethylacetate extract of marine fungi *Aspergillus oryzae*

		(mg kg^{-1})	Increase in paw volume (mL)				
Treatment	Dose	0 h	1 h	2 h	3 h	4 h	5 h
Control	-	0.584±0.008	0.927±0.009	0.982±0.007	0.937±0.005	0.849±0.007	0.712±0.007
Diclofenac sodium	10	0.599±0.007ns	0.765±0.004***	0.742±0.007***	0.643±0.008***	0.615±0.008***	0.595±0.008***
A. oryzae	100	0.594±0.014ns	0.840±0.008***	0.705±0.005***	0.648±0.007***	0.608±0.006***	0.581±0.007***
	200	0.588±0.009ns	0.814±0.008***	0.703±0.009***	0.640±0.005***	0.601±0.005***	0.587±0.006***

***$p<0.001$ (significant), n = 4, values are mean±SEM

Table 2: Analgesic activity of ethylacetate extract of marine fungi *Aspergillus oryzae*

		Reaction time (sec) after drug administration			Percentage inhibition (%)		
Treatment	Dose (mg kg^{-1})	1 h	2 h	3 h	1 h	2 h	3 h
Control	-	1.75±0.48	2.75±0.25	2.5±0.29	0.0	0.0	0.0
Diclofenac sodium	100	6.5±0.65***	9.25±0.48***	12.25±0.48***	73.07	70.20	79.59
A. oryzae	100	5.75±0.48***	6.75±0.48***	8.5±0.29***	69.57	59.25	70.59
	200	6.5±0.29***	8.75±0.63***	9.5±0.65***	73.07	68.57	73.68

N=4, values are Mean±SEM ***$P<0.001$ (significant)

Table 3: Antipyretic activity of ethylacetate extract of marine fungi *Aspergillus oryzae*

Treatment	Dose (mg kg^{-1})	Initial temp. (°C)	Rectal temperature °C in hour± SEM				
			0 h	1 h	2 h	3 h	4 h
Control	-	37.53±0.09	38.15±0.16	38.16±0.09	38.09±0.09	37.98±0.09	37.89±0.1
Paracetamol	45	37.59±0.23	38.20±0.17	37.96±0.14ns	37.78±0.14ns	37.72±0.14ns	37.59±0.11*
A. oryzae	100	37.34±0.12	38.00±0.06	37.78±0.08*	37.67±0.09*	37.59±0.1ns	37.41±0.11ns
	200	37.50±0.05	38.20±0.07	37.93±0.04ns	37.75±0.04ns	37.68±0.03**	37.52±0.04*

Mean±SEM, (n=4), ns – non significant, *p<0.05, **p<0.01 (significant)

Table 4: CNS Stimulant activity of ethylacetate extract of marine fungi *Aspergillus oryzae*

Treatment	Dose (mg kg^{-1})	Mean locomotor activity scores in ten minutes			
		Before treatment	After treatment	Percentage of locomotor activity	Nature of action
Control	-	163.5±1.708	162.5±1.708	0.6	-
Caffeine	30	170.5±2.218	224±3.163	24	Stimulant
A. oryzae	100	175.5±1.708	218±20.897	19.5	Stimulant
	200	188.3±1.315	190.5±2.5	1.15	-

Mean±SEM, (n=4)

Table 5: Anti ulcer activity of ethylacetate extract of marine fungi *Aspergillus oryzae*

Treatment	Dose (mg kg^{-1})	Volume of gastric juice	pH	Free acidity (meq L^{-1})	Total acidity (meq L^{-1})	Ulcer scores (Ul L^{-1})	Serum alkaline phosphate (Ka)	Serum calcium (mg dL^{-1})
Control	-	9.50±0.129	2.40±0.06	96.65±0.221	119±0.183	2.50±0.287	48.81±0.08	9.74±0.041
Ranitidine	13.5	2.75±0.171***	4.89±0.013***	33±0.183***	58.50±0.129***	0.25±0.143***	12.78±0.081***	11.57±0.53***
A. oryzae	100	2.50±0.129***	3.55±0.013***	56.50±0.129***	69.85±0.171***	1±0***	25.41±0.461***	10.66±0.03***
	200	1.60±0.082***	3.95±0.013***	45.35±0.222***	63.50±0.238***	0.75±0.145***	18.44±0.067***	12.05±0.068***

N=4, values are Mean±SEM ***p<0.001 (significant)

Anti ulcer activity: Anti ulcer activity of test extract of *A. oryzae* is summarized in Table 5. The test extracts (100 and 200 mg kg^{-1}) and positive control (Ranitidine) reduction in gastric juice volume was statistically significant ($p<0.001$), when compared with control. Test extract at 100 mg kg^{-1} reduced gastric juice volume almost equally that of positive control. But the extract 200 mg kg^{-1} showed higher reduction when compared to positive control but not statistically significant.

Test extract showed elevation in pH indicating their capacity to reduce the acidity of gastric juice. The mean pH value of extract at 200 mg kg^{-1} (3.95) was almost equal as that of positive control (4.89). Moreover, test extract at 200 mg kg^{-1} (45.35 meq L^{-1}) decreased the gastric free acidity than positive control (33 meq L^{-1}) and total acidity (63.50 meq L^{-1}) also decreased at 200 mg kg^{-1} and observed to be almost equal to positive control (58.50 meq L^{-1}).

The severity of gastric ulceration was assessed based on the mean ulcer index. The extract at 200 mg kg^{-1} exhibited mean ulcer index of 0.75 Ul L^{-1} which was almost comparable to that of positive control (0.25 Ul L^{-1}) and the ulcer score was lower than control (2.50 Ul L^{-1}) indicating anti ulcer activity of the test extract.

Serum calcium level was induced by extract at 200 mg kg^{-1} (12.05 mg dL^{-1}) which was almost equivalent to positive control (11.57 mg dL^{-1}). Similar level decreases in alkaline phosphatase level was also observed for test extract at 200 mg kg^{-1} (18.44 ka) and it was lower than Ranitidine (12.78 ka). This obtained results indicated that test extract at 200 mg kg^{-1} significantly reduced ($p<0.001$) the volume of gastric juice, free and total acidity, alkaline phosphatase and increase calcium level thereby found to be potential against gastric ulcers in rats.

DISCUSSION

Marine environment continuously provides broad and structurally diverse array of pharmacologically active compounds to mankind. These compounds have been shown to exhibit anti cancer, antimicrobial, antifungal, anti-inflammatory and other pharmacological activities[3,24]. Anti-inflammation test is one of the most common and primary test for screening pharmacological properties of natural products. Prolonged inflammation leads to pathogenesis of various diseases such as rheumatoid arthritis, periodontitis, chronic inflammation, otitis, autoimmune diseases, hearing loss and bacterial sepsis[4,5].

In the present observation, ethyl acetate extract of *A. oryzae* significantly reduced the formation of paw edema induced by carrageenan. This study was done for 4 hrs because carrageenan induced hind paw edema in rat includes three distinct phases, such as release of histamine and serotonin in the first phase (0-120 min), kinins in the second phase (180 min) and prostaglandin in the third phase (240 min)[25]. The extract showed marked inhibition in edema formation. This observed inhibition of edema could be attributed to the activation

of the kinin system, accumulation of neutrophils and the release of several mediators such as prostanoids and cytokines[26].

The exhibited anti-inflammatory activity of fungus *A. oryzae* was dose dependent. In a similar study[27] reported anti inflammatory activity of asperlin from marine-derived fungus *Aspergillus* sp. SF-5044. Active extract at 200 mg kg^{-1} showed reduction in paw volume and the activity was relatively higher than standard drug Diclofenac sodium. Similar level activities of fungal metabolites have been reported earlier. For example, Belofsky *et al.*[28] reported that Oxepinamides isolated from tunicate fungi *Acremonium* sp. exhibited comparable higher activity than standard drug. Thus this study suggested that the fungal metabolites could be used as effective drugs in the treatment of inflammation.

Both anti inflammatory and analgesic activities are mediated through a common mechanism. Hence, tail-flick behavior in rat was used to evaluate the analgesic activity of fungal extract which also showed increasing activity with increasing extract concentration but activity was observed to be lower than standard drug. It may be attributed to the pure form of standard drug and crude form of extract. Extract at 200 mg kg^{-1} during 1 h showed almost equal activity to standard drug. This observation of pain killing effect could be attributed to selective modulation of neuronal nicotinic receptors in the spinal cord and brain in rat[29].

Yeast induced pyrexia is a classical method for testing antipyretic activity of any compound. Ethyl acetate extract of *A. oryzae* showed comparable dose dependent antipyretic activity with higher activity at 200 mg kg^{-1} which was equal potent activity to standard drug. This activity of extract could be attributed to the ability of compound in inhibition of prostaglandin biosynthesis[30].

Locomotor activity of rats was stimulated by extract of *A. oryzae.* This observed CNS stimulant activity at 100 mg kg^{-1} was lower when compared with standard drug Caffeine (30 mg kg^{-1}). The possible mechanism of CNS stimulant activity by fungal extract may be attributed to the inhibition of phosphodiesterases or blockade of adenosine receptors or increases in the rate of turnover of norepinephrine and dopamine or increases in the sensitivity of post-synaptic central catecholamine receptors or increases in the brain content in rats[31].

Extract of *A. oryzae* significantly reduced gastric ulcer formation, free acidity, total acidity and ulcer index in pylorus ligation model. Volume of gastric secretion is an important factor in the production of gastric ulcers which believed to be due to stress induced increase in gastric hydrochloric acid secretion and their accumulation in unprotected lumen of stomach. They lead to auto digestion of gastric mucosa and breakdown of gastric mucosal barrier. They also cause upper gastrointestinal damage including lesions, ulcers and life threatening perforation and hemorrhage[32]. In this observation, test extract showed almost equal effect compared to standard drug Ranitidine (13.5 mg kg^{-1}). At 200 mg kg^{-1} test extract significantly reduced the volume of gastric juice, free and total acidity of gastric secretion, alkaline phosphatase and showed increase in calcium level in rats. This obtained anti-ulcer activity of fungal extract suggests that fungal metabolites may have the ability to stimulate mucus, bicarbonate and the prostaglandin secretion and also counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen thereby may inhibit ulcer formation[33].

The present study indicated the efficient nature of fungal metabolites of *A. oryzae* with anti inflammatory, analgesic, antipyretic, CNS stimulant and anti ulcer properties. Thus this work suggests that marine fungi could be used as a source for isolation of diverse compounds with pharmacological activities. Further exploration of this active crude extract would certainly lead to isolation of potentially useful compounds.

REFERENCES

1. Liberra, K. and U. Lindequist, 1995. Marine fungi: A prolific resource of biologically active natural products? Pharmazie, 50: 583-588.
2. Kosta, S., R. Jain and A. Tiwari, 2008. Marine fungi: Potential source of pharmacological compounds. Pharmacologyonline, 1: 1-3.
3. Mayer, A.M.S. and M.T. Hamann, 2005. Marine pharmacology in 2001-2002: Marine compounds with anthelmintic, antibacterial, anticoagulant, antidiabetic, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis and antiviral activities; affecting the cardiovascular, immune and nervous systems and other miscellaneous mechanisms of action. Comp. Biochem. Physiol. Toxicol. Pharmacol., 140: 265-286.
4. Watanabe, K., K. Jinnouchi, A. Hess, O. Michel and T. Yagi, 2001. Detection of apoptotic change in the lipopolysaccharide (LPS)-treated cochlea of guinea pigs. Hear Res., 158: 116-122.
5. Hirose, M., K. Ishihara, A. Saito, T. Nakagawa, S. Yamada and K. Okuda, 2001. Expression of cytokines and inducible nitric oxide synthase in inflamed gingival tissue. J. Periodontol., 72: 590-597.
6. Costa, B., M. Colleoni, S. Conti, D. Parolaro, C. Franke, A.E. Trovato and G. Giagnoni, 2004. Oral anti-inflammatory activity of cannabidiol, a non-psychoactive constituent of cannabis, in acute carrageenan-induced inflammation in the rat paw. Naunyn-Schmiedeberg's Arch. Pharmacol., 369: 294-299.

7. Vane, J.R. and R.M. Botting, 1998. Anti-inflammatory drugs and their mechanism of action. Inflamm. Res., 47: S78-S87.
8. Reddy, D.R.S., A.V. Audipudi, G.D. Reddy and C.V.S. Bhaskar, 2011. Antioxidant, antiinflammatory and antifungal activity of marine sponge *Subergargoria suberosa*-derived natural products. Int. J. PharmTech Res., 3: 342-348.
9. Belofsky, G.N., P.R. Jensen, M.K. Renner and W. Fenical, 1998. New cytotoxic sesquiterpenoid nitrobenzoyl esters from a marine isolate of the fungus *Aspergillus versicolor.* Tetrahedron, 54: 1715-1724.
10. Tsuda, M., T. Mugishima, K. Komatsu, T. Sone and M. Tanaka *et al.*, 2003. Speradine A, a new pentacyclic oxindole alkaloid from a marine-derived fungus *Aspergillus tamarii.* Tetrahedron, 59: 3227-3230.
11. Kato, H., T. Yoshida, T. Tokue, Y. Nojiri and H. Hirota *et al.*, 2007. Notoamides A-D: Prenylated indole alkaloids isolated from a marine-derived fungus, *Aspergillus* sp. Angewandte Chemie Int. Edn., 46: 2254-2256.
12. Fremlin, L.J., A.M. Piggott, E. Lacey and R.J. Capon, 2009. Cottoquinazoline A and Cotteslosins A and B, metabolites from an Australian marine-derived strain of *Aspergillus versicolor.* J. Nat. Prod., 72: 666-670.
13. Li, J.L., P. Zhang, Y.M. Lee, J. Hong, E.S. Yoo, K.S. Bae and J.H. Jung, 2011. Oxygenated hexylitaconates from a marine sponge-derived fungus *Penicillium* sp. Chem. Pharm. Bull., 59: 120-123.
14. Thirunavukkarasu, P., T. Ramanathan, L. Ramkumar and R. Shanmugapriya, 2010. Anti ulcer effect of *Avicenia officinalis* leaves in albino rats. World Applied Sci. J., 9: 55-58.
15. Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drug. Proc. Soc. Exp. Biol. Med., 111: 544-547.
16. Hukkeri, V.I., B.S. Patil, R.V. Savadi and C.V. Nagathan, 2004. Analgesic, antipyretic and diuretic activities of *Basella rubra* Linn. Indian Drugs, 41: 536-539.
17. Jain, P.S., S. Mallipedi, D.P. Belsare, S.C. Mandal and S.C. Pal, 2007. Analgesic activity of stem bark of *Kigelia pinnata* Linn. Indian Drugs, 44: 63-65.
18. Ramasamy, M.S. and S.S. Kumar, 2009. Anti inflammatory, antinociceptive and central nervous system depressant activities of marine bacterial extracts. J. Pharmaco. Toxicol., 4: 152-159.
19. Shay, H., S.A. Komarov, S.S. Fels, D. Meranze, M. Gruenstein and H. Siplet, 1945. A simple method for the uniform production of gastric ulceration in the rat. Gastroenterology, 4: 43-61.
20. Kind, P.R. and E.G. King, 1954. Colorimetric method for determination of serum alkaline phosphatase. J. Clin. Pathol., 7: 322-322.
21. Schwarzenbach, G., 1955. The complexones and their analytical application. Analyst, 80: 713-729.
22. Biggs, H.G. and W.R. Moorehead, 1974. O-Cresolpthalein direct colorimetric method. Clin. Chem., 20: 1458-1460.
23. Kunchandy, J., S. Khanna and S.K. Kulkarni, 1985. Effect of alpha 2 agonists clonidine, guanfacine and B-HT 920 on gastric acid secretion and ulcers in rats. Arch. Int. Pharmacodyn Ther., 275: 123-138.
24. Gul, W. and M.T. Hamann, 2005. Indole alkaloid marine natural products: An established source of cancer drug leads with considerable promise for the control of parasitic, neurological and other diseases. Life Sci., 78: 442-453.
25. Olajide, O.A., J.M. Makinde and S.O. Awe, 1999. Effects of the aqueous extract of *Bridelia ferruginea* stem bark on carrageenan-induced oedema and granuloma tissue formation in rats and mice. J. Ethnopharmacol., 66: 113-117.
26. Akkol, E.K., F.N. Yalcin, D. Kaya, I. Callis, E. Yasilada and T. Ersoz, 2008. *In vivo* anti inflammatory and antinociceptive actions of some *Lamium* species. J. Ethanopharmacol., 118: 166-172.
27. Lee, D.S., G.S. Jeong, B. Li, S.U. Lee, H. Oh and Y.C. Kim, 2011. Asperlin from the marine-derived fungus *Aspergillus* sp. SF-5044 exerts anti-inflammatory effects through heme oxygenase-1 expression in murine macrophages. J. Pharmacol. Sci., 116: 283-295.
28. Belofsky, G.N., M. Anguer, P.R. Jensen, W. Fenical and M. Kock, 2000. Oxepinamides AC and fumiquinazolines H-I: Bioactive metabolites from a marine isolate of a fungus of the genus *Acremonium.* Chemi, Eur. J., 6: 1355-1360.
29. Marwick, C., 1998. Medical news and perspectives. J. Am. Medical. Assoc., 279: 1679-1681.
30. Vane, J.R., 1978. The evaluation of non-steroidal anti-inflammatory drugs and their mechanisms of action. Drugs, 33: 18-27.
31. Misra, A.L., N.L. Vadlamani and R.B. Pontani, 1986. Effect of caffeine on cocaine locomotor stimulant activity in rats. Pharmacol. Biochem. Behav., 24: 761-764.
32. Sachin, S.S. and R.J. Archana, 2009. Antiulcer activity of methanol extract of *Erythrina indica* Lam. leaves in experimental animals. Pharmacogn. Res., 1: 396-401.
33. Borrelli, F. and A.A. Izzo, 2000. The plant kingdom as a source of anti-ulcer remedies. Phytother. Res., 14: 581-591.