Int. J. Biol. Res., 6(2): 73-84, 2018.

IN VITRO ANTI-INFLAMMATORY AND ANTIFUNGAL ACTIVITY OF STEM AND ROOTS EXTRACTS OF *IPOMOEA PES-CAPRAE* (LINN.) R. BR.

Sana Eijaz[1*], Amber Mirza[1], Muneeba Khan[2], Anjum Perveen[2], Shazia Mansuri[2], Muhammad Nadir[3,4], Muneera Naz Baloch[1] and Fouzia Haider[1]

[1]Department of Microbiology, University of Karachi, Karachi, Pakistan
[2]Center for Plant Conservation, University of Karachi, Karachi, Pakistan
[3]Department of Chemistry, University of Karachi, 75270, Karachi, Pakistan
[4]Department of Chemistry, Government Degree Boys College, Sector 5/L, New Karachi, 75850, Karachi, Pakistan
**Corresponding authoremail: sanaeijaz2013@gmail.com*

ABSTRACT

The anti-inflammatory and antifungal activity of different extracts of roots and stem of *Ipomoea pes-caprae* were evaluated. Extracts were prepared in four different solvents i.e. methanol, chloroform, ethyl acetate and hexane using soxhlet apparatus and used in different concentrations. The anti-inflammatory activity was checked by observing the inhibition of albumin denaturation and antifungal activity by well-diffusion assay. The results showed that the methanol and chloroform extract of stem have strong antifungal activity.The plant extracts were potentially effective in suppressing fungal growth with variable potency. In case of roots, chloroform and ethyl acetate extracts were more effective and showed inhibition of large number of the tested fungi. Chloroform extract of stem and methanol extract of roots showed maximum inhibition of albumin denaturation. Among all the tested fungal isolates *Mucor, Aspergillus niger, Aspergillus flavus*, showed sensitivity to almost all of the extracts of roots and stem.

KEYWORDS: *Ipomoea pes-caprae*, extracts, fungi, inflammation, albumin denaturation

INTRODUCTION

The implementation of herbal medicine was as far as 60,000 years ago in Iraq and 8000 years ago in China which has been indicated by archaeological studies. The parts of plant which are utilized in herbal therapy include seeds, berries, roots, leaves, fruits, bark, flower and even the whole plant (Pan *et al*., 2014). In both underdeveloped and developed countries, the desire for plant based therapies is growing because of the increasing awareness that they are natural products, non-narcotic, simply biodegradable, having no injurious side effects and easily accessible at affordable prices (Aruna and Nandakishore, 2014). Indiscriminate use of antibiotics has become major factor responsible for the emergence and dissemination of multidrug resistance strains of microorganisms. Wide spread emergence of resistance to the newly admitted antimicrobial agents indicate that new families of antimicrobial agents have short duration time and because of this researcher moving their attention towards herbal products (Khan *et al*., 2009).

Ipomoea pes-caprae is broadly distributed as pioneer species on tropical beaches worldwide, emerging just above the high tide line. It is present at many construction sites and empty lots in coastal villages and also common on both lime stone and on

consolidated beaches, on calcareous and quartz sand (Devall, 1992).The most important biologically active constituents, present in *Ipomoea pes-caprae*, are ergoline alkaloids, indolizidine alkaloids, nortropane alkaloids, phenolics compounds, coumarins, diterpene, isocoumarin, benzenoids, flavonoids, antocianosides, glycolipids, lignin and triterpenes. Due to their content of ergot type alkaloids, several species of *Ipomoea* are used as hallucinogenic (Meira *et al*., 2011). *Ipomoea pes-caprae* is used as a suspension for urinary and kidney problems, hypertension, skin infections, digestive disorders, menstrual disorders, lumbago, dysentery, arthritis, rheumatism and as an anti-flatulance agent. *Ipomoea pes-caprae* also used to treat bedsores and orally it is used to cure rubella as well as to alleviate jelly fish sting pruritis (Martinez *et al.,* 2014; Anandhi and Ushadevi, 2013). In *Ipomoea pes-caprae,* fats, proteins, crude fiber, essential fatty acids i.e., omega3 and omega6 are present (Anandhi and Ushadevi, 2013).

Aspergillus species are environmental saprophytes present in both outdoor environments i.e. soil and plant debris as well as indoor environments i.e. hospitals. In case of immunocompromised or debilitated patients, *Aspergillus* species can be lethal (Mirhendi *et al*., 2015). *Aspergillus flavus* is the second major cause of invasive and non-invasive aspergillosis in humans and animals after *Aspergillus fumigates* (Yu *et al*., 2005). *A.flavus* also cause endocarditis, reno-uretic aspergilloma in diabetic patients, pulmonary and disseminated infection in patients with falciparum malaria, primary CNS aspergillosis and aortic graft infections (Krishnan *et al*., 2008). *Asgergillus niger* cause several cases of otitis externa and invasive pulmonary aspergillosis in immunocompromised patients. *A.niger* also present in tissue samples with painful black gangrenous appearance (Pawer and Thaker, 2006; Zmeili and Soubani, 2007).

Mucor is ubiquitous in nature and different from other Zygomycetes that it has the property of dimorphism (Hermet *et al*., 2012; Orlawski, 1991). *Mucor* species cause Mucoromycosis in immunocompromised patients such as bone marrow transplanted patients and associated with high mortality rate (Schwarz *et al*., 2006). *Penicillium* is one of the most important fungi present in the diverse range of habitat from soil to vegetation, air, indoor environment and various food products (Visagie *et al*., 2014). *Penicillium* species are rarely associated with the diseases, however it mostly cause infections in AIDS patients (Vanittanakom *et al*., 2006). *Saccharomyces* is ascomycetous yeast and found everywhere (Munoz *et al*., 2005). *S.cerevasiae* cause vaginitis and cutaneous to systemic blood stream infections (Perez-Torrado and Querol, 2016).

Candida species have been recognized as the forth main cause of nosocomial infections. *Candida* has the ability to induce a wide variety of infections ranging from superficial to deep seated mycoses (Sida *et al*., 2015). *Microsporium gypseum* is the most commonly isolated keratinophilic fungi found in the soil (Gugnani *et al*., 2014). *M.gypseum* causes Tineacorporis, Tineacapitis, kerion and Tineabarbae (Romano *et al* 2008). *Trichophyton mantagrophyte* is a ubiquitous dermatophyte (Kim *et al*., 2001). *T.mantagrophytes* cause kerion which is a painful and suppurative plaque associated with purulent drainage and regional lymphadenopathy (Isa-Isa *et al*., 2010). *T.mantagrophytes* can cause infections in both humans and animals (Frealle, *et al*., 2007).

Inflammation is the process linked with tissue damage and in which various chemical mediators are produced. These mediators are strongly associated with several diseases including autoimmune and heart diseases, cancer, arthritis, diabetes etc. By reducing the inflammation process, these diseases may be treated. A number of medicines are available for this but still there is a need to design more drugs (Hunter, 2012; Chen *et al*., 2018; Govindappa *et al*., 2011).

MATERIAL AND METHOD

Plant collection: *Ipomoea pes-caprae* (Linn.) R. Br. (stem and roots) was collected from coastal area of Karachi and the voucher specimen has been submitted in the Karachi University Herbarium (G.H.no.86616). The plant was identified by the taxonomist of Herbarium, Center for Plant Conservation which is situated in the University of Karachi.

Extracts preparation: For the preparation of plant extracts, different solvents were used including methanol, ethyl acetate, hexane and chloroform. After collection, the plant was washed, dried, crushed and grinded to convert into powdered form. The soxhlet apparatus was used to make different extracts of the collected plant. Fifteen g of powdered plant material was weighed and wrapped in whatsman 41 filter paper. The plant material was placed inside the extraction tube, over which a condenser was fixed. The soxhlet apparatus was connected to a distillation flask and a chiller which was set at temperature 5˚C. One hundred and fifty mL of respective solvent i.e. hexane, chloroform, ethyl acetate and methanol was poured in the distillation flask placed on a heating mantle. The temperature was adjusted in accordance with the solvent used (30˚C-40˚C). The extraction process was continued for about 14 h. The extract was then transferred to a round bottom flask to concentrate using BUCHI Rota-vapour R-200. The flask containing the extract sample was submerged in a water bath set to the temperature 40˚C. The concentrated extract was partitioned in the extract tube and left opened for the removal of any residual solvent and redissolved in DMSO (di-methyl sulphoxide) to attain the required concentrations i.e. 250µg/50µL, 750µg/50µL, 1500µg/50µL, 3000µg/50µL and 4000µg/50µL and 5000µg/50µL. All the extracts were kept at 4˚C during the experiments (Redfern *et al.*, 2014).

***In vitro* anti-inflammatory activity (Inhibition of albumin denaturation):** The reaction mixture was comprised of test extracts and 1% aqueous solution of bovine albumin fraction. Extracts were used in 200ug concentration as aspirin was used. All the samples were incubated at 37^{o}C for 20 minutes and then heated at 51^{o}C for 20 minutes. After cooling, the turbidity of the samples was measured using spectrophotometer at 660nm. Percent inhibition of protein denaturation was calculated (Govindappa *et al*., 2011) by applying the formula i.e. % inhibition= [{Abs control- Abs sample}/Abs control] x 100, (Abs control = the absorbance without sample, Abs sample = the absorbance of sample extract/standard).

Collection and identification of fungal isolates: The fungal isolates were collected from the microbial culture bank, department of Microbiology and were identified by the help of morphological and colonial characteristics. Lactophenol cotton blue was used to see the morphological characteristics and different media were used to observe the colonial characteristics.

Preparation of fungal suspension: Sabouraud's dextrose agar was used in order to analyze the antifungal activity of different extracts. 0.5 McFarland's index was prepared to get the inoculum size of 1.5 x 10^{8}CFU/ml (Coyle, 2005).

Antifungal effect of different extracts: 0.1ml of the respective fungal suspension was added on the media and lawns were prepared with the help of sterile cotton swab. The plates were left for 30 minutes for proper absorption of fungal culture and wells were made on each plate with the help of a borer of size 8mm (Bell and Grundy, 1968). 50µl of each extract concentrations i.e. 250µg (5%), 750µg (15%), 1500µg (30%), 3000µg (60%), 4000µg (80%) and 5000µg (100%)was added in the respective wells. Extract concentrations were selected after performing MIC assay. 50µl of DMSO was added in one well as a negative control (Al-Bakri and Afifi, 2007; Nostro*et al.*, 2000) and 50µl of nystatin in another well as a positive control. The media plates were incubated at room temperature for 48 hours to one week. The plates were observed for the zone of inhibition around the wells and the diameter was measured in millimeters (mm).

Statistical analysis: Statistical analysis was performed by using the software IBM SPSS Statistics 23. One way analysis of variance (ANOVA) followed by Bonferroni post hoc test and student's t-test were performed to compare the groups with level of confidence $p<0.05$; (where * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$). Data are presented as mean ± SEM.

RESULTS

The present study was performed to analyze the antifungal and anti-inflammatory activity of roots and leaves extracts of *Ipomoea pes-caprae*. The fungi included *Aspergillus flavus, Aspergillus niger, Candida species, Mucor species, Saccharomyces cerevisae, Penicillium* species, *Trichophyton mentagrophytes* and *Microsporum gypseum.* Different concentrations of each extract was prepared in DMSO i.e. 250µg, 750µg, 1500µg, 3000µg, 4000µg and 5000µg for the antifungal activity. DMSO was used as a negative control and nystatin was used as a positive control to compare the activity of various extracts (Table 1).

Effect of stem extracts on fungal isolates

Chloroform extract: When the fungal cultures were treated with chloroform extract of *Ipomoea pes-caprae* stem, no zone of inhibition was observed in case of *Penicillium, Saccharomyces, Microsporium gypseum* and *Trichophyton mantagrophyte.* Significant difference was observed in case of *Penicillium* and *Saccharomyces* at all concentrations when compared with positive control i.e. nystatin ($p<0.05$). In case of *Mucor,* significant difference was observed at 250µg ($p<0.05$) while at other concentrations, non-significant difference was observed as compared to nystatin. *Aspergillus niger* showed significant difference when nystatin compared with all test concentrations ($p<0.05$). On the other hand, highly significant difference ($p<0.001$***) was observed in the zone of inhibition in case of *Aspergillus flavus* and *Candida* when compared with the zone of nystatin (Table 2).

Methanol extract: Methanol extract of *Ipomoea pes-caprae* stem showed that this extract can affect the growth of *Penicillium, Mucor, Saccharomyces, Candida, Microsporium gypseum* and *Trichophyton mantagrophyt*e. Highly significant difference were observed in case of *Mucor, Saccharomyces, Candida and Trichophyton mantagrophyte* while in case of *Microsporium gypseum* highly significant zone was observed at 250µg, 750µg, 1500µg and 3000µg when it was compared to nystatin ($p<0.001$). At 4000µg, less significant difference was observed ($p<0.01$**) and at 5000µg, non-significant difference was observed. No zone of inhibition was observed in case of *Aspergillus niger* and *Aspergillus flavus* at all concentrations (Table 3).

Table 1. Zone of inhibition against nystatin.

Fungi	Nystatin (zone of inhibition, mm)
Aspergillus flavus	4mm
Aspergillus niger	5mm
Candida	4.5mm
Microsporum gypseum	6mm
Mucor	3.5mm
Penicillium	4.5mm
Saccharomyces	4mm
Trichophyton mentagrophytes	3mm

Table 2. Sensitivity of the test organisms to chloroform extract of *Ipomoea pes-caprae* stem (Means of triplicate zones of inhibition in mm ± SEM).

	(Concentration in µg)					
	250	**750**	**1500**	**3000**	**4000**	**5000**
Aspergillus niger	1±00	1±00	1±00	1±00	1±00	1±00
Aspergillus flavus	1±00	1±00	1±00	1±00	1±00	1.3±0.2
Mucor	1.2±0.1	3.8±1.3	3.5±0.8	5.1±1.6	2.3±0.3	7.3±0.4
Penicillium	00±00	00±00	00±00	00±00	00±00	00±00
Saccharomyces	00±00	00±00	00±00	00±00	00±00	00±00
Candida	1.3±0.2	1.2±0.2	1±00	1±00	1±00	1±00
Microsporium gypseum	00±00	00±00	00±00	00±00	00±00	00±00
Trichophyton mantagrophyte	00±00	00±00	00±00	00±00	00±00	00±00

Table 3. Sensitivity of the test organisms to methanol extract of *Ipomoea pes-caprae* stem (Means of triplicate zones of inhibition in mm ± SEM).

	(Concentration in µg)					
	250	**750**	**1500**	**3000**	**4000**	**5000**
Aspergillus niger	00±00	00±00	00±00	00±00	00±00	00±00
Aspergillus flavus	00±00	00±00	00±00	00±00	00±00	00±00
Mucor	1.5±0.3	1.5±0.3	1±00	1±00	1.2±0.2	10.6±0.6
Penicillium	1±00	1±00	1±00	1±00	1±00	1±00
Saccharomyces	1±00	1±00	1±00	1±00	1±00	1.3±0.2
Candida	1.5±0.3	1±00	1.2±0.2	1±00	1±00	2±0.3
Microsporium gypseum	00±00	00±00	0.6±0.3	1±00	2.1±0.3	3.5±0.3
Trichophyton mantagrophyte	1±00	00 ±00	00±00	2±0.3	1.8±0.2	2.3±0.2

Ethyl acetate extract: When we analyzed the effect of ethyl acetate extract of *Ipomoea pes-caprae* stem, the results showed that this extract exhibit observable activity against *Aspergillus niger, Aspergillus flavus* and *Penicillium.*While in case of *Mucor, Saccharomyces, Candida, Microsporium gypseum* and *Trichophyton* no zone of inhibition was observed (Table 4).

Table 4. Sensitivity of the test organisms to ethyl acetate extract of *Ipomoea pes-caprae* stem (Means of triplicate zones of inhibition in mm ± SEM).

	(Concentration in µg)					
	250	**750**	**1500**	**3000**	**4000**	**5000**
Aspergillus niger	1±00	1±00	1±00	1±00	1±00	1±00
Aspergillus flavus	1±00	1±00	1±00	1±00	1±00	1±00
Mucor	00±00	00±00	00±00	00±00	00±00	00±00
Penicillium	1±00	1±00	1±00	1±00	1±00	1±00
Saccharomyces	00±00	00±00	00±00	00±00	00±00	00±00
Candida	00±00	00±00	00±00	00±00	00±00	00±00
Microsporium gypseum	00±00	00±00	00±00	00±00	00±00	00±00
Trichophyton mantagrophyte	00±00	00±00	00±00	00±00	00±00	00±00

Table 5. Sensitivity of the test organisms to hexane extract of *Ipomoea pes-caprae* stem (Means of triplicate zones of inhibition in mm ± SEM).

	(Concentration in µg)					
	250	**750**	**1500**	**3000**	**4000**	**5000**
Aspergillus niger	00±00	00±00	00±00	00±00	00±00	00±00
Aspergillus flavus	00±00	00±00	00±00	00±00	00±00	2±0.5
Mucor	00±00	00±00	00±00	00±00	1±00	1±00
Penicillium	00±00	00±00	00±00	00±00	00±00	00±00
Saccharomyces	00±00	00±00	00±00	00±00	00±00	1±00
Candida	00±00	00±00	00±00	00±00	00±00	00±00
Microsporium gypseum	00±00	00±00	00±00	00±00	00±00	00±00
Trichophyton mantagrophyte	00±00	00±00	00±00	00±00	00±00	00±00

Hexane extract: The results of hexane extract of *Ipomoea pes-caprae* showed that in case of *Penicillium*, *Aspergillus flavus*, *Aspergillus niger*, *Candida*, *Microsporium gypseum*, *Trichophyton mantagrophyte* and *Saccharomyces*, no zone of inhibition was found at any concentration of the extract. In case of *Mucor*, zone of inhibition was observed at 4000µg and 5000µg and *Aspergillus flavus* showed zone of inhibition at 5000µg only (Table 5).The highest zone of inhibition (mm) of *Ipomoea pes-caprae* stem extracts against different fungal isolates shown in Fig.1.

Effect of roots extracts on fungal isolates

Chloroform extract: When the chloroform extract of *Ipomoea pes-caprae* roots was examined, it was observed that the zone of inhibition appeared at all concentrations of the extract against *Aspergillus niger*, *Aspergillus flavus* and *Mucor*. *Penicillium* showed sensitivity at 5000µg only, *Saccharomyces* at 1500ug, 3000ug, 4000ug and 5000ug and *Candida* at 3000ug, 4000ug and 5000ug. No zone of inhibition was observed in case of *Microsporium gypseum* and *Trichophyton mantagrophyte* (Table 6).

Table 6. Sensitivity of the test organisms to chloroform extract of *Ipomoea pes-caprae* roots (Means of triplicate zones of inhibition in mm ± SEM).

	(Concentration in µg)					
	250	**750**	**1500**	**3000**	**4000**	**5000**
Aspergillus niger	1.2±0.2	3±0.3	1.3±0.3	2.2±0.4	1.8±0.4	1.5±00
Aspergillus flavus	1±00	0.6±0.3	1±00	1.8±0.2	1±00	1.8±0.2
Mucor	0.6±0.3	1±00	1.2±0.2	1.3±0.3	1.5±0.3	1.8±0.4
Penicillium	00±00	00±00	00±00	00±00	00±00	2.3±0.4
Saccharomyces	00±00	00±00	0.6±0.3	0.6±0.3	1±00	1±00
Candida	00±00	00±00	00±00	0.6±0.3	0.6±00	00 ±00
Microsporium gypseum	00±00	00±00	00±00	00±00	00±00	00±00
Trichophyton mantagrophyte	00±00	00±00	00±00	00±00	00±00	00±00

Table 7. Sensitivity of the test organisms to methanol extract of *Ipomoea pes-caprae* roots (Means of triplicate zones of inhibition in mm ± SEM).

	(Concentration in µg)					
	250	**750**	**1500**	**3000**	**4000**	**5000**
Aspergillus niger	1±00	1±00	1±00	1±00	1±00	1.2±0.2
Aspergillus flavus	0.7±0.3	1±00	1±00	1±00	1±00	1±00
Mucor	00±00	00±00	00±00	00±00	00±00	00±00
Penicillium	2±0.5	2.2±0.2	1.8±0.4	3.2±0.3	3±0.3	3.3±0.4
Saccharomyces	00±00	00±00	00±00	00±00	00±00	00±00
Candida	00±00	00±00	00±00	00±00	00±00	00±00
Microsporium gypseum	00±00	00±00	00±00	00±00	00±00	00±00
Trichophyton mantagrophyte	00±00	00±00	00±00	00±00	00±00	00±00

Methanol extract: The methanolic extract of *Ipomoea pes-caprae* roots affects the growth of *Penicillium, Aspergillus niger* and *Aspergillus flavus. Saccharomyces, Candida, Microsporium gypseum and Trichophyton mantagophyte* remained insensitive to all the concentrations of the extract as no zone of inhibition was observed (Table 7).

Ethyl acetate extract: When we examined the ethyl acetate extract of *Ipomoea pes-caprae* roots, no zone of inhibition was observed against *Penicillium, Trichophyton mantagrophyte* and *Microsporium gypseum*. When *Aspergillus niger* was treated with the extract, non-significant difference was observed between nystatin and all the test concentrations of the extract and in case of *Aspergillus flavus,* good zones were observed at 3000ug, 4000ug and 5000ug.Visible zones were observed against *Mucor* and *Saccharomyces* at all the concentrations of the extract. *Candida* did not show sensitivity at 250µg, 750µg and 1500µg concentrations while at 3000µg, 4000µg and 5000µg zones were observed (Table 8).

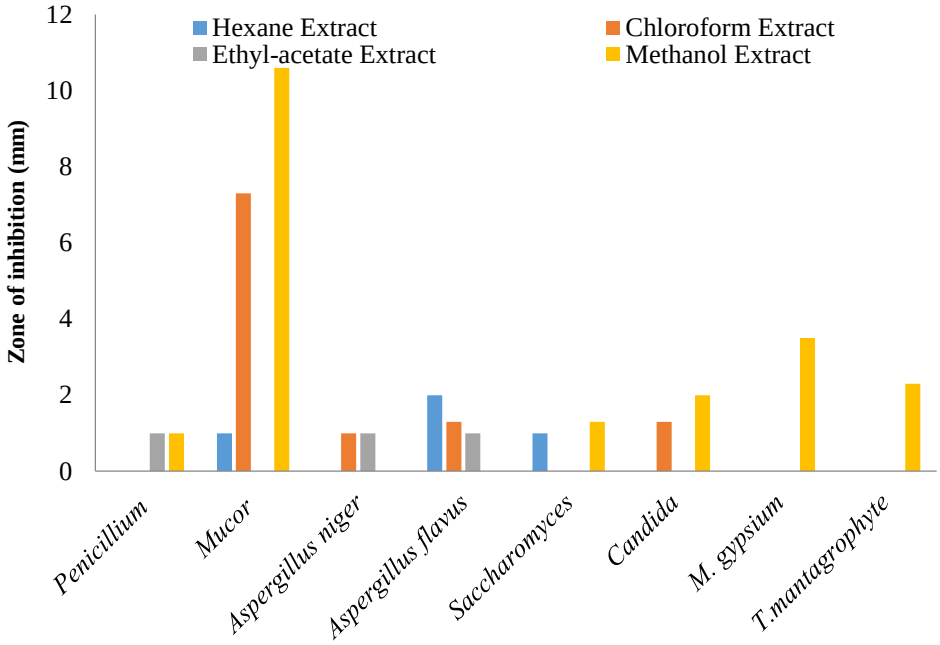

Fig. 1. Zone of inhibition (mm) of *Ipomoea pes-caprae* stem extracts against different fungal isolates.

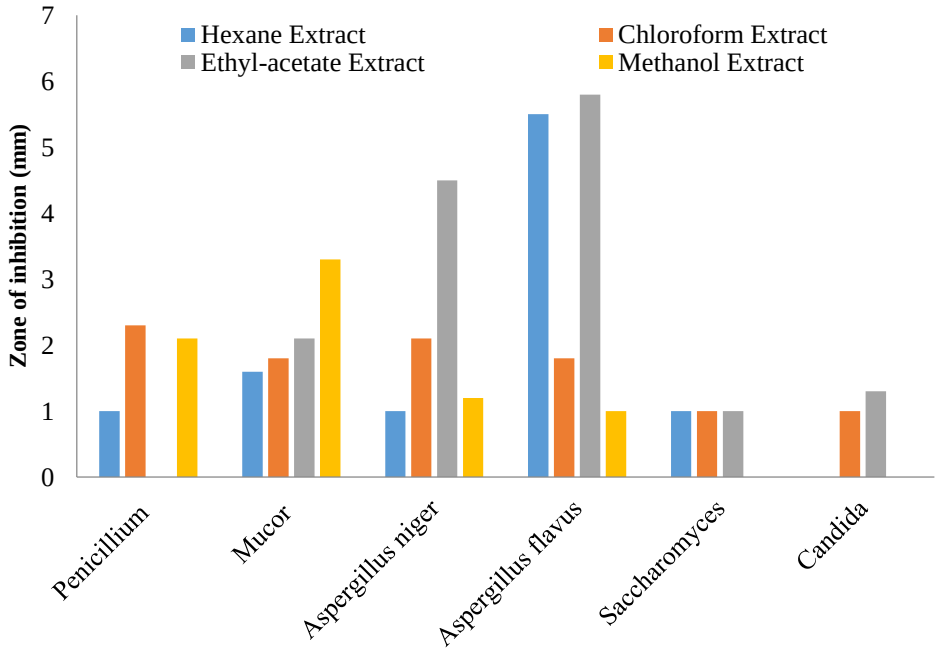

Fig. 2. Zone of inhibition (mm) of *Ipomoea pes-caprae* roots extracts against different fungal isolates.

Table 8. Sensitivity of the test organisms to ethyl acetate extract of *Ipomoea pes-caprae* roots (Means of triplicate zones of inhibition in mm ± SEM).

	(Concentration in µg)					
	250	**750**	**1500**	**3000**	**4000**	**5000**
Aspergillus niger	3.7±0.9	2.1±0.4	3.1±0.7	4.5±0.8	3.3±0.7	4.3±0.6
Aspergillus flavus	3.7±0.3	3.2±0.3	3.7±0.6	5.8±0.4	5.5±0.3	5.3±0.2
Mucor	1.5±00	1.7±0.4	1.3±0.3	2.2±0.3	2±0.3	2.2±0.3
Penicillium	00±00	00±00	00±00	00±00	00±00	00+00
Saccharomyces	1±00	0.7±0.4	0.6±00	1±00	1±00	1±00
Candida	00±00	00±00	00±00	0.7±0.3	1.3±0.2	0.7±0.3
Microsporium gypseum	00±00	00±00	00±00	00±00	00±00	00±00
Trichophyton mantagrophyte	00±00	00±00	00±00	00±00	00±00	00±00

Table 9. Sensitivity of the test organisms to hexane extract of *Ipomoea pes-caprae* roots (Means of triplicate zones of inhibition in mm ± SEM).

	(Concentration in µg)					
	250	**750**	**1500**	**3000**	**4000**	**5000**
Aspergillus niger	1±00	1±00	1±00	1±00	1±00	1±00
Aspergillus flavus	2±0.5	1.3±0.7	3.1±0.8	4.3±0.4	5.5±0.3	3.3±0.2
Mucor	1±00	1±00	1±00	1±00	1±00	1.6±0.2
Penicillium	00±00	0.6±0.3	1±00	0.6±0.3	0.6±0.3	1±00
Saccharomyces	0.6±0.3	00±00	00±00	00±00	0.6±0.3	1±00
Candida	00±00	00±00	00±00	00±00	00±00	00±00
Microsporium gypseum	00±00	00±00	00±00	00±00	00±00	00±00
Trichophyton mantagrophyte	00±00	00±00	00±00	00±00	00±00	00±00

Hexane extract: To evaluate the effect of hexane extract of *Ipomoea pes-caprae* roots, same method was followed. When the fungal cultures were treated with six different concentrations of extract, i.e. 250µg, 750µg, 1500µg, 3000µg, 4000µg, 5000µg, it was observed that *Penicillium*, *Mucor*, *Aspergillus niger* and *Saccharomyces s*howed statistically significant zones of inhibition. In case of *Aspergillus flavus*, significant zone was observed at 750µg ($p<0.05$*) while at other concentrations i.e. 250µg, 1500µg, 3000µg, 4000µg, 5000µg non-significant zone was observed as compared to nystatin. No zone was found in case of *Candida*, *Microsporium gypseum* and *Trichophyton mantagrophyte* (Table 9). The highest zone of inhibition (mm) of *Ipomoea pes-caprae* roots extracts against different fungal isolates shown in Fig.2.

Anti-inflammatory activity

Inhibition of albumin denaturation: A leading cause of inflammation is the denaturation of proteins. We analyzed the effect of different extracts of stem and roots of *Ipomoea pes-caprae* on protein denaturation. Our results showed that few of our extracts were effective in inhibiting heat induced albumin denaturation (Table 10). Maximum inhibition was observed by chloroform extract of stem and methanol extract of roots i.e. 50.12 and 56.57 respectively. However, other extracts did not show observable inhibition. Aspirin was used as a standard anti-inflammatory drug (Govindappa *et al*., 2011) and showed the maximum inhibition i.e. 75.79 at of 200µg concentration.

Table 10. Effect of different solvent extracts of *Ipomoea pes-caprae* on albumin denaturation (Means of turbidity at 660nm ± SEM).

Extracts	Stem (O.D)	Roots (O.D)
Chloroform (200µg/mL)	50.12±0.06	30.31±0.06
Methanol (200µg/mL)	30.22±0.06	56.57±0.08
Hexane (200µg/mL)	25.21±0.07	30.33±0.09
Ethyl acetate (200µg/mL)	26.00±0.09	20.45±0.03
Aspirin (200µg/mL)	75.79±0.08 (standard)	

DISCUSSION

In this study, the anti-inflammatory and antifungal activity of *Ipomoea pes-caprae* was evaluated. Different fungal isolates were treated with four types of solvents. Hexane, chloroform, ethyl acetate and methanol were used by keeping the point in mind that different solvents have different solubility for polar and non-polar compounds so that we could analyze the antifungal effect of various compounds present in the experimental plant. The fungal isolates used were *Penicillium, Mucor, Aspergillus niger, Aspergillus flavus, Microsporum gypseum, Trichophyton mantagrophyte, Candida* and *Saccharomyces cerevisae* by using well diffusion method. The anti-inflammatory activity of various extracts was studied and compared to the standard anti-inflammatory drug aspirin.

On the basis of zone of inhibition produced by *Ipomoea pes-caprae* against different fungal isolates, the order of inhibitory potential of stem was observed as methanol extract > chloroform extract > hexane extract > ethyl acetate extract. In case of roots, the highest zone of inhibition against different fungal isolates was found as ethyl acetate extract > hexane extract > methanol extract > chloroform extract.

Collectively, the result showed that the methanol extract of stem of *Ipomoea pes-caprae* was more effective as maximum number of fungi showed sensitivity to these extracts. In case of methanol and chloroform extracts, larger zones of inhibition were observed against *Mucor* at 5000ug concentration as compared to the zone of nystatin against *Mucor.*The zones observed as 10.6mm and 7.3mm, respectively. Hexane and ethyl acetate extracts of stem were less effective against the fungal isolates.

Among all the root extracts, chloroform and ethyl acetate extracts showed inhibition of large number of the tested fungi. Ethyl acetate extract showed considerable inhibition of *Aspergillus flavus* and *Aspergillus niger* at all the concentrations of extract and the zones of inhibition were larger and nearer to the zone appeared in case of nystatin. Hexane extract of roots showed substantial inhibition of *Aspergillus flavus* at 250µg, 1500µg, 3000µg, 4000µg, 5000µg concentrations and the inhibition was almost similar to the inhibition of nystatin.

Chloroform extract of stem and methanol extract of roots showed maximum inhibition of albumin denaturation i.e. 50.12 and 56.57, respectively. However, other extracts did not show observable inhibition. Aspirin was used as a standard anti-inflammatory drug (Govindappa *et al*., 2011) and showed the maximum inhibition i.e. 76.89 at 200µg concentration.

Among all the tested fungal isolates, *Mucor, Aspergillus niger* and *Aspergillus flavus* showed sensitivity to almost all of the extracts of roots and stem of *Ipomoea pes-caprae* whereas *Penicillium, Saccharomyces* and *Candida* showed susceptibility to the least number of extracts. *Microsporum gypseum* and *Trichophyton mantagrophyte* were only susceptible to the methanol extract of stem of *Ipomoea pes-caprae.*

CONCLUSION

This study showed that the extracts of *Ipomoea pes-caprae* exhibited antifungal and anti-inflammatory activity. It was observed by our experiments that methanol extract of *Ipomoea pes- caprae* stem and ethyl acetate extract of *Ipomoea pescaprae* roots showed strong inhibition of almost all the fungal isolates. The results revealed that the plant extracts were potentially effective in suppressing fungal growth with variable potency. Moreover, chloroform extract of stem and methanol extract of roots showed observable anti-inflammatory activity.

REFERENCES

Al-Bakri, A.G. and F.U. Afifi.(2007). Evaluation of antimicrobial activity of selected plant extracts by rapid XTT colorimetry and bacterial enumeration. *J. Microbiol.Meth*., 68: 19-25.

Anandhi, K. and T. Ushadevi.(2013). A study on antioxidant, proximate analysis, antimicrobial activity, phtochemical analysis of *Ipomeapes-caprae*.*Int. J. Biotech. Allied Fields*, 1: 380-386.

Aruna, D.S. and D.L.V. Nandakishore.(2014). Haritaki a boon to herbalism- a review.*Scholars Acad. J. Biosci*., 2: 132-136.

Bell, S.C. and W.E. Grundy.(1968). Preparation of agar wells for antibiotic assay.*Applied Microbiology*, 16: 1611-1612.

Chen, L., D. Huidan, C. Hengmin, F. Jing, Z. Zhicai, D. Junliang, L. Yinglun, W. Xun and Z. Ling. (2018). Inflammatory responses and inflammation-associated diseases in organs.*Oncotarget*, 9: 7204-7218.

Coyle, M.B. (2005). Manual of antimicrobial susceptibility testing.American Society for Microbiology.

Devall, M.S. (1992). The Biological Flora of Coastal Dunes And Wetlands. 2. *Ipomoea Pes-Caprae*(L.) Roth. *J. Coast Res*., 8: 442-456.

Frealle, E., M. Rodrigue, N. Gantois, C. Aliouat, E. Delaporte, D. Camus, E. Dei-Cas, C. Kauffmann-Lacroix, J. Guillot and L. Delhaes. (2007). Phylogenetic analysis of Trichophytonmantagrophyte human and animal isolates based on MnSOD and ITS sequence comparison.*Microbiology*, 153: 3466-3477.

Govindappa, M., T.S. Sadananda, R. Channabasava and V. B. Raghavendra. (2011). *In vitro* anti-inflammatory, lipoxygenase, xanthine oxidase andacetycholinesterase inhibitory activity of *Tecomastans*(L.) Juss.exKunth. *Int. J. Pharma. Bio. Sci*., 2: 275-285.

Gugnani, H.C., S. Sharma and K. Wright.(2014). A preliminary study on the occurrence of keratinophilic fungi in soils of Jamaica.*Rev. Inst. Med. Trop. Sao Paulo*.,56: 231-234.

Hermet, A., D. Mhesust, J. Mountier, G. Barbier and J. Jany.(2012). Molecular systematics in the genus Mucor with special regards to species encountered in cheese.*Fungal Mycol.*, 116: 692-705.

Hunter, P. (2012). The inflammation theory of disease.*EMBO Reports*, 13: 968-970.

Isa-Isa, R., R. Arenas and M. Isa. (2010). Inflammatory tineacapitis: kerion, dermatophytic granuloma, and mycetoma. *Clin.Dermatol.*, 28: 133-136.

Khan, R., B. Islam, M. Akram, S. Shakil, A. Ahmad, S.A. Manazir, M. Siddiqui and A.U. Khan.(2009). Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin.*Molecules*, 14: 586-597.

Kim, J.A., Y. Takahashi, R. Tanaka, K. Fukushima, K. Nishimura and M. Miyaji.(2001). Identification and subtyping of Trichophytonmantagrophyte by random amplified polymorphic DNA.*Mycoses*, 44: 157-165.

Krishnan, S., E.K. Manavathu and P.H. Chandrasekar. (2008). Aspergillusflavus: an emerging non-fumigatus*Aspergillus species* of significance. *Mycoses*, 52: 206-222.

Martinez, E., M. Ameen, D. Tejada and R. Arenas. (2014). *Microsporium*spp. onycomycosis: disease presentation, risk factors and treatment responses in an urban population. *Braz. J. Infect. Dis.*, 18: 181-186.

Meira, M., E.D.S. Pereira, J.M. David and J.P. David. (2011). Review of the genus Ipomoea: traditional uses, chemistry and biological activities. *Braz. J. Pharmacog*., 22: 682-713.

Mirhendi, H., F. Zarei, M. Motamedi and S. Nouripour-Sisakht. (2016). Aspergillustubingensis and *Aspergillusniger* as the dominant black Aspergillus, use of simple PCR-RFLP for preliminary differentiation. *J. Mycol. Med*., 592: 1-8.

Munoz, P., E. Bouza, M. Cuenca-Estrella, J.E. Marıa, M.P. Jesus, M. Sanchez-Somolinos, C. Rincon, J. Hortal and T.Pelaez. (2005). *Saccharomyces cerevasiae*fungemia: an emerging infectious disease. *Clinic Infect. Dis*., 40: 1625-1634.

Nostro, A., M.P. Germano, V. Dangelo, A. Marino and M.A. Cannatelli.(2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity.*Lett. Appl.Microbiol*., 30: 379-384.

Orlowski, M. (1991).Mucor dimorphism.*Microbiol. Rev*., 55: 234-258.

Pan, S., G. Litscher, S. Gao, S. Zhou, Z. Yu, H. Chen, S. Zhang, M. Tang, J. Sun and K. Ko. (2014). Historical perspective of traditional indigenous medical practices: The current renaissance and conservation of herbal resources. *Evid.Based Complem.Alternant. Med.*, 14: 1-20.

Pawar, V.C. and V.S. Thaker. (2006). In vitro efficacy of 75 essential oils against Aspergillusniger. *Mycoses.* 49: 316-323.

Perez-Torrado R and A.Querol. (2016). Opportunistic Strains of *Saccharomyces cerevisiae*: A Potential Risk Sold in Food Products. *Frontiers inMicrobiol.*, 6: 1-5.

Redfern, J., M. Kinninmonth, D. Burdass and J. Verran. (2014). Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. *J. Microbiol. Biol. Educ*., 15: 45-46.

Romano, C., L. Massai, A. Gallo and M. Fimiani.(2008). *Microsporiumgypseum*infection in the Siena area in 2005-2006.*Mycoses*, 52: 67-71.

Schwarz, P., S. Bretagne, J. Gantier, D. Hermoso, O. Lortholary, F. Dromer and E. Dannaoui.(2006). Molecular identification of zygomycetes from culture and experimentally infected tissues.*J. Clinic. Microbiol*.,44: 340-349.

Sida, H., P. Shah, J. Pethani, L. Patel and H. Shah.(2015). Study of biofilm formation as a virulence marker in *Candida* species isolated from various clinical specimens.*Int. J. Med. Sci. Public Health*, 5: 1-5.

Vanittanakom, N., C.R. Cooper, M.C. Fisher and T. Sirisanthana.(2006). *Penicilliummarneffi*infection and recent advances in the epidemiology and molecular biology aspects.*Clinic.Microbiol. Rev*., 19: 95-110.

Visagie, C.M., J. Houbraken, J.C. Frisvad, S.B. Hong, C.H.W. Klaassen, G. Perrone, K.A. Seifert, J. Varga, T. Yaguchi and R.A. Samson. (2014). Identification and nomenclature of the genus *Penicillium. Stud. Mycol*., 78: 343-371.

Yu, J., T.E. Cleveland, W.C. Nierman and J.W. Bennett. (2005). .*Aspergillusflavus*genomics: Gateway to human and animal health, food safety, and crop resistance to diseases. *Rev. Iberoam. Micol*., 22: 194-202.

Zmeili, O.S. and A.O. Soubani. (2007). Pulmonary aspergillosis: a clinical update. *Q. J. Med*., 100: 317-334.

(Received 3 August 2018; Accepted 27 October 2018)