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Research Article

An investigation of the antimicrobial activity of the aqueous, dichloromethane, ethanol and methanol extract of the seeds and whole plant of *Ipomoea nil*

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Ipomoea nil (Linn) *Roth*, (morning glory / kaladanah; *Convolvulaceae*), is frequently grown in gardens and lawns for its ornamental flowers and often runs wild in hedges and wastelands. Plant is documented to possess beneficial effect in erectile dysfunction, impotence and also have antispasmodic, bronchodilator, blood purifier, diuretics, astringent, anti-inflammatory and hepato-protective etc. In this study, disc diffusion method was adopted to evaluate and compare the antimicrobial activity of the aqueous and organic (DCM, ethanol, methanol) extracts of the seeds and whole plant (leaves, flower and bark) of Ipomoea nil, in order to know the best extract and plant part having the beneficial activity against pathogenic bacteria species of both G +ve strains, i.e., *Staphylococcus aureus, Bacillus pumilus, Streptococcus pneumoniae,* G -ve strains, i.e., *Escherichia coli, Citrobacter freundii* and *Klebsiella pneumoniae* and two species of fungi (*Candida albicans, Aspergillus niger*). Methanolic and ethanolic extract of the seeds of the *Ipomoea nil* showed significant antibacterial activity against *Staphylococcus aureus* (G +ve) having the relative percentage of inhibition of 89.88 % and 85.67 % respectively, while methanolic extract of the whole plant also have relative percentage of inhibition of 83.96%, whereas ethanolic extract of the whole plant have good antibacterial activity. Aqueous and DCM extract of seeds and whole plant showed weak inhibitory response. Modified agar well diffusion method was adopted to measure the minimum inhibitory concentration (MIC) and MIC values for G +ve, lies within the range of 30 to 60 µg /ml (seeds), 60 to 90 µg /ml (whole plant) and for the Gram –ve, it lies within the range of 90 to 250 µg /ml (seeds), 180 to 500 µg /ml (whole plant) and for the fungi, it varies from 500 to 2000 µg /ml. Present study clearly indicate that the antimicrobial activity varies from part to part and the plant material used and it also indicate that the methanolic extract of *Ipomoea nil* is a potentially good candidate for the therapy of antibacterial-resistant bacteria and would therefore require further study.

Keywords: *Ipomoea nil*, Methanol Extract, Ethanol Extract, Dichloromethane Extract, Antimicrobial Activity, Disc Diffusion Method, Minimum Inhibitory Concentration.

1. INTRODUCTION

For the human and animal health care, natural products of the plant origin have been used and are being used throughout the world. Mitigation of drug resistance and treatment of infectious diseases are the great challenges. However, due to inappropriate use of synthetic antibiotics, certain strains of fungi and bacteria developed the ability to produce the substances which block the action of antibiotics and change their target to penetrate through various processes such as, conjugation, transduction and transformation (Shirazi et al., 2007; Jain et al., 2010). This drives the discovery of novel antimicrobial therapeutic agents from the medicinal herbs (Gootz, 1990). It has been explored that medicinal flora of Pakistan comprises nearly 1,650 predominantly tropical

species of family *Convolvulaceae* (Zia et al., 2011). The genus *Ipomoea* has approximately 500-600 species (Austin, 1998), among them twenty species are found in Pakistan (Nasir and Ali, 1995). Plants of this genus have different agricultural, nutritional, ritual and medicinal properties, since immemorial time. In our research study, we only focused on *Ipomoea nil* (Linn) *Roth*, referred by multiple Synonyms, i.e., *Ipomoea hederaceae* (L.) *Jacq*, *Convolvulus nil*, *Convolvulus hederaceae* L, and *Ipomoea githaginea* and is known by vernacular name of morning glory, woolly-morning glory (as flowers wilt after one day), ivy morning glory, habbub-nil (due to blue color of its corolla, nil in Pakistan is used for the blue color), kaladanah (due to black color of the seeds, kala means black and dana means seed). *Ipomoea nil* is frequently grown in gardens and lawns for its ornamental flowers and often runs wild in hedges and wastelands (Nadkarni, 1985; Kritikar and Basu, 2000). Its flower is funnel shaped (2 inch wide) with five hairy sepals which are generally blue, but can show traces of purple, magenta, or white. Each flower is replaced by a 3-celled rounded capsule containing 4–6 seeds. The rather large seeds are brown to black and wedge-shaped. They have a dull surface (Bhattacharjee, 2004). Its flowering period is from June to October and main part used is seed. Its native use is to manage multiple ailments including abdominal diseases i.e., constipation, dyspepsia, and flatulence, respiratory diseases, i.e., bronchitis (Duke et al., 2002). A number of pharmacological properties such as, anthelmintic, blood purifier pungent, diuretics, astringent and anti-inflammatory actions has been described to this plant, besides its use to treat fever, headache, gout, dry the phlegm (Duke et al., 2002; Khare, 2007). The seeds are rubbed on the male genitals to treat erectile dysfunction and on female genitals for lubrication purposes and to increase sexual desire (Joshi, 2000). Paste of seeds is applied topically for cosmetic purposes as it removes dry skin and freckles. Furthermore it is claimed to be associated with weight loss (Chopra et al., 1986). It is used for treatment of diabetes (Bhardwaj et al., 2010). It also exhibit hepato-protective activity (Devi et al., 2010). Its antioxidant activity have been studied by following method i.e., 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, ferric reducing antioxidant power (FRAP) assay, total antioxidant activity and Folin-Ciocalteau reagent assay (Rehman et al., 2011). Recent study has shown that it exhibit inhibitory effect on the secretion of interleukin-8 (IL-8) and generation of reactive oxygen species (ROS) as a means of reported anti-inflammatory and anti-ulcerative properties (Zaidi et al., 2012). The present study was designed to evaluate and compare the antimicrobial activity of the crude extract of the seeds and whole plant of *Ipomoea nil*, prepared from dichloromethane (DCM), ethanol, methanol and distilled water (aqueous extract), against G +ve strains, i.e., *Staphylococcus aureus, Streptococcus pneumoniae* and *Bacillus pumilus,* G -ve strains, i.e., *Escherichia coli, Citrobacter freundii and Klebsiella pneumoniae* and two species of fungi (*Candida albicans and Aspergillus niger*).

2. MATERIAL AND METHODS

2.1 Collection of seeds and plant

Dried seeds of *Ipomoea nil* (500 gm) were purchased from the local market of Multan, (Pakistan) whereas whole plant (leaves, flower and bark) was collected from the Botanical Garden of Bahauddin Zakariya University, Multan and were authenticated by Professor Dr. Altaf Dasti, taxonomist and head of herbarium of Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan (Pakistan) vide voucher number P. Fl. 575 – 8. Fresh *Ipomoea nil* (leaves, flower and bark) was collected in the due free morning and were dried under the shade for the 3 weeks. At the time of collection, weight of the fresh *Ipomoea nil* (leaves, flower and bark) was almost 500 gm, but after drying under shade, it got reduced to 420 gm.

2.2 Extract preparation

Electrical blender was used to prepare the coarse powder (# 40) of *Ipomoea nil* seed and dried whole plants (leaves flower and bark). Extraction was carried out by using the different organic solvents (depending upon their polarity), by adopting the triple maceration (Farooq, 2013). Extract was prepared by soaking the coarse powdered material in a measured volume of dichloromethane, in two separate macerating bottles and agitated at 120 rpm/min for 72 hrs

in rotary orbital shaker, at room temperature. This procedure was repeated further two times with dichloromethane. After maceration, filtration of the soaked coarse powdered material was carried out through muslin cloth (double layered), in order to remove vegetative debris and the obtained filtrate was subsequently filtered through a Whatman-1 filter paper. The filtrates were stored in amber glass air-tight bottles. The extraction of marc was carried out with ethanol, methanol and then with distilled water by adopting same procedure. Rotary evaporator (Rotavapor, BUCHI labrotechnik AG, Model 9230, Switzerland) attached with a vacuum pump and a recirculation chiller was used to concentrate the organic extract (dichloromethane, ethanol and methanol), under reduced pressure at 37 °C, whereas concentration of the aqueous extract was carried out by the lyophilization. Dichloromethane, ethanol, methanol and aqueous extract of seeds were named as InDs.Cr, InEs.Cr, InMs.Cr and InAqs.Cr whereas upper mentioned extract of whole plant of *Ipomoea nil* were named as InDw.Cr, InEw.Cr, InMw.Cr and InAqw.Cr respectively and all extracts are stored in air tight amber glass bottles in refrigerator at −4 °C.

2.3 Extract solution preparation

In vitro, experiments were performed by dissolving 0.15 gram of the crude extract in 0.1ml (100 µl) of 100% dimethylsulfoxide (DMSO) and volume was made up to 1 ml (1000 µl) with distill water to prepare 0.15 g /ml, w/v stock solution (150 mg/ml), due to its insolubility in distilled water and stored in refrigerator at −4 °C (Hussain et al., 2013). The dimethylsulfoxide alone did not show any biological and physiological activity. Thereafter dilution of stock solution (containing 150 mg/ml) was made, to obtain 75 mg/ml concentration which was used for the antimicrobial sensitivity test.

2.4 Determination of phytochemical constituents

Crude methanolic extracts of seeds (InMs.Cr) and whole plant (InMw.Cr) of *Ipomoea nil* was subjected to phytochemical screening for the detection of alkaloids, carbohydrates, tannins, saponins, anthraquinones, steroids and flavonoids as possible important constituents of the plant, according to standard method (Farooq, 2013). Molisch's, benedict's and fehling's tests were performed for the detection of carbohydrates. Appearance of yellowish brown coloration on mixing of Dragendorff's reagent with HCl treated aqueous plant extract solution, conform the presence of alkaloids in extract. The plant material was deemed positive for flavonoids when it gave a yellow color with AlCl3 reagent. Formation of froth on vigorous shaking of the aqueous extract solution, conform the presence of saponins. Development of blue green or dark green coloration on mixing of aqueous FeCl3 with extract solution indicated presence of phenols and tannins. Legal test and Keller-killiani test were performed for the detection of cardiac glycosides. The appearance of pink, violet or red coloration on exposure to NH4OH of the mixture of benzene with aqueous solution of plant extract already acidified with 1% HCl was taken as presence of anthraquinones among the plant constituents. Lieberman burchard test was performed for the steroidal constituents.

2.5 Standard antibiotic discs and culture used

Standard antibiotics discs i.e., flucloxacillin, vancomycin, ceftriaxone, ciprofloxacin, ceftriaxone, and levofloxacin, were used against *Staphylococcus aureus, Bacillus pumilus, Streptococcus pneumoniae, Citrobacter freundii, Escherichia coli* and *Klebsiella pneumoniae* respectively, while against *Candida albicans* and *Aspergillus niger*, amphotericin-B discs were used. All standard discs having drug concentration of 10µg/disc (Oxobid Ltd. Basingstoke, Hampshire, England) were purchased from G. M, Scientific shop, Multan, Pakistan. Whereas all the microorganisms i.e., *Staphylococcus aureus, Bacillus pumilus, Streptococcus pneumoniae, Citrobacter freundii, Escherichia coli, Klebsiella pneumoniae, Candida albicans* and *Aspergillus niger,* were obtained from the University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. All microbes were cultured overnight in a nutrient agar (pH 5) containing agar (1.2%), peptone (0.5%), yeast (0.3%), and NaCl (0.8%) (Cruickshan et al., 1975). Microbial colonies were transferred from fresh culture plates to tube containing 10 ml of nutrient broth media, in order to prepare the inoculums. The tubes were shaken occasionally

for aeration to promote the microbial growth and were incubated overnight at 37°C.

2.6 Determination of antimicrobial activity

Antibacterial activity was determined by standard disc diffusion method (Taylor et al., 1955; Newall et al., 1996; Hussain et al., 2013). Three types of discs were used, i.e., discs containing plant crude extract were used as sample discs, discs containing standard antibiotics were used as positive control, and discs containing the DMSO were used as negative control. The round discs having the size of 6 mm in diameter were prepared from the whatman-1 filter paper by punch machine.

Nutrient agar media and sabouraud dextrose agar media prepared in distilled water and sterilized in autoclave at 121°C for 15 minutes, having pressure of 15 lb/inch^2. Pour the media into separate petri dishes and allowed to set as a firm gel on cooling. The thickness of gels layer should range between 2-3 mm. The test petri-dishes were incubated overnight at 37°C and those showing no growth of any kind were selected for further work. The bacteria and fungi were transferred from inoculums to petri-dishes by using flame-sterilized forceps, which were subsequently spread by streaking method. The petri-dish with these test discs were then incubated inverted condition for 24 hours at 37°C. At the end of the incubation period, zone of inhibition (mm) of the each extract were measured in comparison with the positive control (Andrews, 2001; Khyade and Vaikos, 2011). For the conformation of the results, each test was performed in triplicate.

2.6.1 Calculation of relative percentage inhibition

The relative percentage inhibition of the crude extract with respect to positive control was calculated by using the following formula (Ajay et al., 2002; Hussain et al., 2013).

$$\text{Relative percentage inhibition of crude extract} = 100 \times (a - b) / (c - b)$$

Where,

a: total area of inhibition of the test extract

b: total area of inhibition of the solvent

c: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using

$$\text{Area of zone of inhibition} = \pi r^2$$

Where,

"r" is radius of zone of inhibition

2.6.2 Determination of minimum inhibitory concentration (MIC)

Modified agar well diffusion method was adopted for the determination of the MIC value of the methanolic crude extract of seeds and whole plant of *Ipomoea nil* (Saratha et al., 2010). The crude extracts were dissolved in DMSO to obtain a concentration range of 30, 60, 90, 180, 250, 500, 1000, 1500, and 2000 µg/ml. In each of these plates four wells were cut out using a cork borer. Using a micropipette, 100 µl of each dilution was added in to wells and plates were incubated at 37ºC for 24 hours. The minimum concentration of each extract showing a clear zone of inhibition was considered to be MIC.

2.7 Statistical analysis

The results of the antimicrobial activity of crude extract are expressed as mean ± standard deviation of the response of triplicates determinations per sample. Statistically significant differences between groups were measured using one-way analysis of variance (ANOVA) followed by two sample *t-test* of all groups versus their respective control group and $*p < 0.05$ was considered statistically significant, $p > 0.05$ was considered as non-significant and $**p < 0.01$ was considered highly significant. Results were analyzed statically by using "Graph pad Prism" version 6, (Graph Pad Software, San Diego, CA, USA).

3. RESULTS

3.1 Phytochemical screening

Freshly prepared methanolic extracts of seeds and whole plant of *Ipomoea nil* were subjected to a preliminary phytochemical screening for various constituents and their results are depicted in Table 1.

Table 1: Phytochemical screening of methanolic extract of seeds and whole plant of *Ipomoea nil*

Chemical tests	InMs.Cr	InMw.Cr	Chemical tests	InMs.Cr	InMw.Cr
Test for carbohydrates			**Test for tannin**		
A. Molisch's test	Positive	Positive	**A.** FeCl3 test		
B. Fehling's test			**B.** Acetic acid test	Positive	Positive
C. Benedict's test			**C**. KmnO4 test		
Test for alkaloids			**Test for cardiac glycosides**		
A. Hager's test					
B. Wagner's test	Positive	Positive	**A.** Legal test	Negative	Negative
C. Dragendorff's test			**B.** Keller-killiani test		
Test for flavonoids			**Test for anthraquinone**		
A. Lead acetate test	Positive	Positive	**A.** Borntraggers's test	Negative	Negative
B. Ferric chloride test			**B.** Modified Borntraggers's test		
Test for saponins			**Test for steroids**		
Foam test	Positive	Positive	Lieberman burchard test	Positive	Positive
Test for protein			**Test for coumarin**		
A. Biuret test	Positive	Positive	**A.** Alkaline reagent test	Negative	Negative
B. Lead acetate test			**B.** NaOH soaked paper test		

Where, InMs.Cr = Methanolic crude extract of seeds of *Ipomoea nil;* InMw.Cr = Methanolic crude extract of whole plant of *Ipomoea nil.*

3.2 In Vitro antimicrobial activity

Diameter of the zone of inhibition, relative percentage of inhibition and (MIC) of the DCM, ethanolic, methanolic and aqueous extract of the seeds and whole plant (leaves, flower and bark) of the *Ipomoea nil*, against different pathogenic bacteria and fungi, are shown in Table 2, 3, 4 and 5. Crude methanolic extract of the seed of the *Ipomoea nil*, showed stronger antibacterial activity against studied G +ve bacterial strains as compared to G -ve strains and fungal species, in comparison with crude methanolic extract of whole plant and DCM, ethanolic and aqueous extract of seeds and whole plant of the *Ipomoea nil*.

The methanolic crude extract of seeds (InMs.Cr) showed the diameter of the zone of inhibition (mm) (including diameter of disc 6 mm) of 20.15 against *Bacillus pumilus*, 21.45 against *Staphylococcus aureus*, 19.75 against *Streptococcus pneumoniae*, 20.30 against *Escherichia coli*, 18.45 against *Citrobacter freundii* and 16.25 against *Klebsiella pneumoniae* as compared with standard drug vancomycin (22.29), flucloxacillin (22.65), ceftriaxone (22.50), ceftriaxone (23.55), ciprofloxacin (22.36) and levofloxacin (21.70) with relative percentages of inhibition 81.76, 89.88, 77.10, 74.30, 68.10 and 56.10 respectively.

Similarly, the ethanolic crude extract of seeds (InEs.Cr) showed the diameter of the zone of inhibition (mm) (including diameter of disc 6 mm) of 19.95 against *Bacillus pumilus*, 20.95 against *Staphylococcus aureus*, 19.00 against *Streptococcus pneumoniae*, 19.55 against *Escherichia coli*, 17.45 against *Citrobacter freundii* and 15.90 against *Klebsiella pneumoniae* with relative percentages of inhibition 80.15, 85.67, 71.32, 68.90, 61.15 and 53.70 respectively whereas the DCM extract of seeds (InDs.Cr) showed the diameter of the zone (mm) of inhibition of 17.25 against *Bacillus pumilus*,

18.10 against *Staphylococcus aureus*, 17.15 against *Streptococcus pneumoniae*, 17.95 against *Escherichia coli*, 15.25 against *Citrobacter freundii* and 13.95 against *Klebsiella pneumoniae* with relative percentages of inhibition 59.92, 63.90, 58.10, 58.90, 46.10 and 41.35 respectively.

The methanolic crude extract of the whole plant of the *Ipomoea nil* (InMw.Cr) showed the diameter of the zone of inhibition (mm) (including diameter of disc 6 mm) of 19.00 against *Bacillus pumilus*, 20.75 against *Staphylococcus aureus*, 18.55 against *Streptococcus pneumoniae*, 19.25 against *Escherichia coli*, 17.00 against *Citrobacter freundii* and 15.50 against *Klebsiella pneumoniae*, with relative percentages of inhibition 72.70, 83.96, 67.95, 66.82, 57.80 and 51.10 respectively. Similarly, the ethanolic crude extract of the whole plant of the *Ipomoea nil* (InEw.Cr) showed the diameter of the zone of inhibition (mm), (including diameter of disc 6 mm) of 18.45 against *Bacillus pumilus*, 19.95 against *Staphylococcus aureus*, 17.70 against *Streptococcus pneumoniae*, 18.40 against *Escherichia coli*, 16.15 against *Citrobacter freundii* and 14.60 against *Klebsiella pneumoniae* with relative percentages of inhibition 68.55, 77.62, 61.90, 61.10, 52.17 and 45.30 respectively whereas the DCM extract of the whole plant of the *Ipomoea nil* (InDw.Cr) showed the diameter of the zone of inhibition (mm) of 16.95 against *Bacillus pumilus*, 17.45 against *Staphylococcus aureus*, 16.65 against *Streptococcus pneumoniae*, 16.95 against Escherichia coli, 14.90 against *Citrobacter freundii* and 13.00 against *Klebsiella pneumoniae* with relative percentages of inhibition 57.58, 59.40, 54.77, 58.70, 44.40 and 35.90 respectively.

Aqueous extract of the seeds and whole plant of *Ipomoea nil* showed the minimum inhibitory response as compared to the DCM, ethanolic and methanolic extract of the seeds and whole plant of *Ipomoea nil*. Whereas, DCM, methanolic, ethanolic and aqueous extract of both seeds and whole plant showed the weak inhibitory response against fungal species i.e., *Candida albicans* and *Aspergillus niger*, in comparison with the amphotericin-B.

After statistical analysis, P value was determined which was found to be significant for methanolic and ethanolic extract, against G +ve, i.e., less than 0.05 ($P < 0.05$). It shows that methanolic and ethanolic extract has excellent antibacterial activity against Gram +ve strains as compared to Gram –ve strain of bacteria, while weak response against fungal species.

Table 2: Zone of inhibition of the DCM, ethanolic, methanolic and aqueous extract of the seeds of *Ipomoea nil* against different strains of bacteria and fungi

Microbes	Zone of Inhibition (mm/sensitive strain)						
	Crude Extract of the seeds of *Ipomoea nil*				Positive control		
	InDs. Cr[a]	InEs.Cr[a]	InMs.Cr[a]	InAqs.Cr[a]	(10 µg/disc)		-ve
B. pumilus	17.25	19.95	20.15	14.95	Vancomycin	22.29	_
S. aureus	18.10	20.95	21.45	16.55	Flucloxacillin	22.65	_
S. pneumoniae	17.15	19.00	19.75	15.45	Ceftriaxone	22.50	_
E. coli	17.95	19.55	20.30	16.10	Ceftriaxone	23.55	_
C. freundii	15.25	17.45	18.45	13.95	Ciprofloxacin	22.36	_
K pneumoniae	13.95	15.90	16.25	11.95	Levofloxacin	21.70	_
C. albicans	13.65	16.10	16.70	10.15	Amphotericin-B	20.35	_
A. niger	14.60	16.55	17.40	12.20	Amphotericin-B	21.85	_

Values are presented as mean of triplicate experiments, [a]= Diameter of the zone of inhibition including

diameter of disc 6mm, -ve = negative control, i.e., Dimethylsulfoxide, +ve control = standard drug discs,- = no response; InDs.Cr = crude extract of the seeds of *Ipomoea nil* in DCM, InEs.Cr = crude extract of the seeds of *Ipomoea nil* in ethanol, InMs.Cr = crude extract of the seeds of *Ipomoea nil* in methanol, InAqs.Cr = Aqueous crude extract of the seeds of *Ipomoea nil*.

Table 3: Zone of inhibition of the DCM, ethanolic, methanolic and aqueous extract of the whole plant of the *Ipomoea nil* against different strains of bacteria and fungi

Microbes	Zone of Inhibition (mm/sensitive strain)						
	Crude Extract of the whole plant of the *Ipomoea nil*				Positive control		
	InDw.Cr[a]	InEw.Cr[a]	InMw.Cr[a]	InAqw.Cr[a]	(10 µg/disc)		-ve
B. pumilus	16.95	18.45	19.00	13.20	Vancomycin	22.29	_
S. aureus	17.45	19.95	20.75	14.95	Flucloxacillin	22.65	_
S. pneumoniae	16.65	17.70	18.55	13.95	Ceftriaxone	22.50	_
E. coli	16.95	18.40	19.25	14.20	Ceftriaxone	23.55	_
C. freundii	14.90	16.15	17.00	12.20	Ciprofloxacin	22.36	_
K pneumoniae	13.00	14.60	15.50	9.90	Levofloxacin	21.70	_
C. albicans	12.80	14.95	15.95	9.00	Amphotericin-B	20.35	_
A. niger	13.95	15.75	16.15	10.75	Amphotericin-B	21.85	_

Values are presented as mean of triplicate experiments, [a]= Diameter of the zone of inhibition including diameter of disc 6mm, -ve = negative control, i.e., Dimethylsulfoxide, positive control = standard drug discs, - = no response.; InDw.Cr = crude extract of the whole plant of the *Ipomoea nil* in DCM, InEw.Cr = crude extract of the whole plant of the *Ipomoea nil* in ethanol, InMw.Cr = crude extract of the whole plant of the *Ipomoea nil* in methanol, InAqw.Cr = Aqueous crude extract the whole plant of the *Ipomoea nil*

Table 4: Relative percentage inhibition of DCM, ethanolic, methanolic and aqueous extract of the seeds and whole plant of *Ipomoea nil* against different strains of bacteria and fungi

Microbes	Relative percentage inhibition (%)							
	InDs.Cr	InEs.Cr	InMs.Cr	InAqs.Cr	InDw.Cr	InEw.Cr	InMw.Cr	InAqw.Cr
B. pumilus	59.92	80.15	81.76	45.05	57.85	68.55	72.70	35.10
S. aureus	63.90	85.67	89.88	53.43	59.40	77.62	83.96	43.60
S. pneumoniae	58.10	71.32	77.10	47.20	54.77	61.90	67.95	38.45
E. coli	58.90	68.90	74.30	46.75	58.70	61.10	66.82	36.36
C. freundii	46.52	61.15	68.10	38.95	44.40	52.17	57.80	29.80
K pneumoniae	41.35	53.70	56.10	30.33	35.90	45.30	51.10	20.82
C. albicans	45.00	62.60	67.35	24.90	39.60	54.00	61.55	19.60
A. niger	44.65	57.40	63.24	31.20	40.80	52.00	54.65	24.20

InDs.Cr = crude extract of the seeds of *Ipomoea nil* in DCM, InEs.Cr = crude extract of the seeds of *Ipomoea nil* in ethanol, InMs.Cr = crude extract of the seeds of *Ipomoea nil* in methanol, InAqs.Cr = Aqueous crude extract of the seeds of *Ipomoea nil*, InDw.Cr = crude extract of the whole plant of the

Ipomoea nil in DCM, InEw.Cr = crude extract of the whole plant of the *Ipomoea nil* in ethanol, InMw.Cr = crude extract of the whole plant of the *Ipomoea nil* in methanol, InAqw.Cr = Aqueous crude extract the whole plant of the *Ipomoea nil*.

3.3 Minimum inhibitory concentration

As shown in Table 5, the methanolic crude extract of seeds (InMs.Cr) showed strong inhibition against tested G +ve bacteria with MIC value of 30 µg/ml, while 90 µg/ml for the G-ve bacteria. Whereas, MIC values for the ethanolic extract of seeds (InEs.Cr) were ranged from 60-180 µg/ml. For the methanolic and ethanolic extract of the whole plant of *Ipomoea nil*, MIC values were ranged from 60-250 and 90-500 µg/ml respectively. MIC values for the fungal species were ranged from 500-2000 µg/ml.

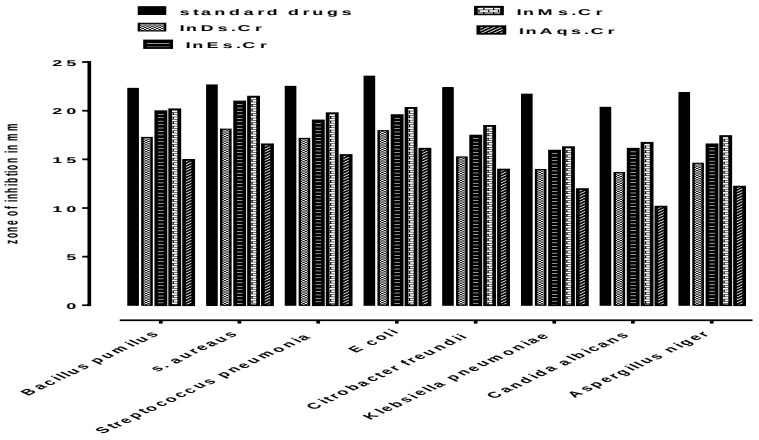
In this study, methanolic crude extract of seeds (InMs.Cr) showed the highest antibacterial activity against studied G +ve bacteria with lowest MIC values of 30 µg/ml.

Table 5: Minimum inhibition concentration (MIC) against different strains of bacteria and fungi

Bacterial strains	**Minimum inhibitory concentration(µg/ml)**			
	Seeds		Whole Plant	
	InMs.Cr	InEs.Cr	InMw.Cr	InEw.Cr
B. pumilus	30	60	60	90
S. aureus	30	60	60	90
S. pneumoniae	30	60	60	90
E. coli	90	180	180	250
C. freundii	90	180	180	500
K pneumoniae	90	250	250	500
C. albicans	500	1000	1500	2000
A. niger	500	1000	1500	2000

InMs.Cr = crude extract of the seeds of *Ipomoea nil* in methanol, InEs.Cr = crude extract of the seeds of *Ipomoea nil* in ethanol, InMw.Cr = crude extract of the whole plant of the *Ipomoea nil* in methanol, InEw.Cr = crude extract of the whole plant of the *Ipomoea nil* in ethanol.

(A)



(B)

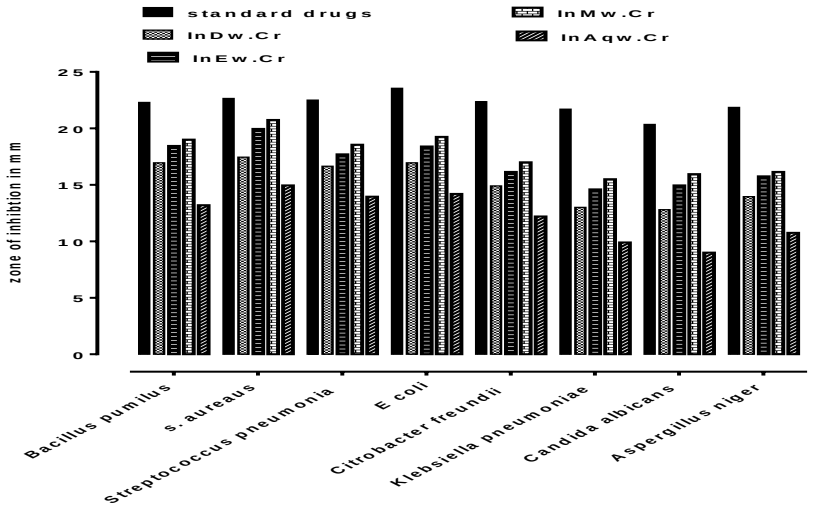
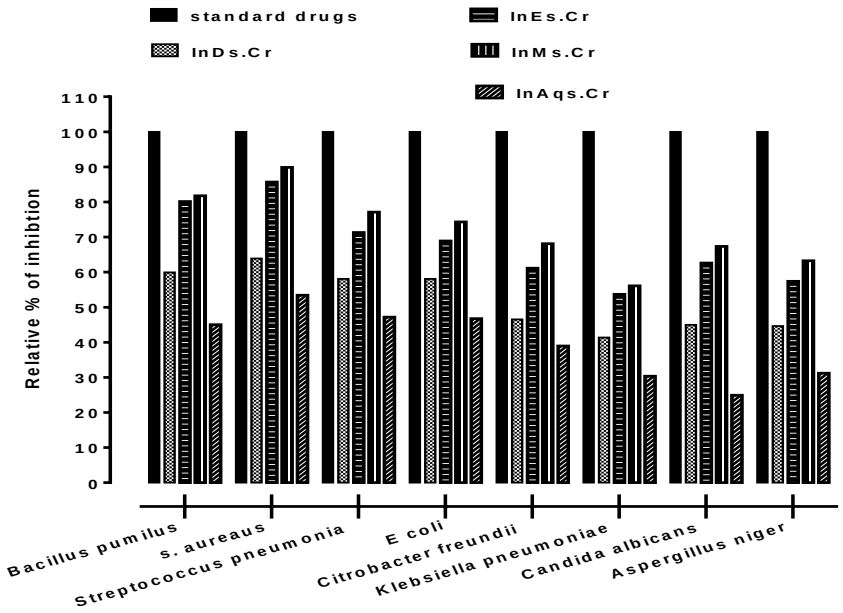


Figure 1: Zone of inhibition of the DCM, ethanolic, methanolic and aqueous extract of the (A) seeds and (B) whole plant of the *Ipomoea nil* in diameter (mm) against different strains of bacteria and fungi (values are expressed as mean ± SEM., n = 3).; InDs.Cr = crude extract of the seeds of the *Ipomoea nil* in DCM, InEs.Cr = crude extract of the seeds of the *Ipomoea nil* in ethanol, InMs.Cr = crude extract of the seeds of the *Ipomoea nil* in methanol, InAqs.Cr = Aqueous crude extract the seeds of the *Ipomoea nil*. InDw.Cr = crude extract of the whole plant of the *Ipomoea nil* in DCM, InEw.Cr = crude extract of the whole plant of the *Ipomoea nil* in ethanol, InMw.Cr = crude extract of the whole plant of the *Ipomoea nil* in methanol, InAqw.Cr = Aqueous crude extract the whole plant of the *Ipomoea nil*.

(A)



(B)

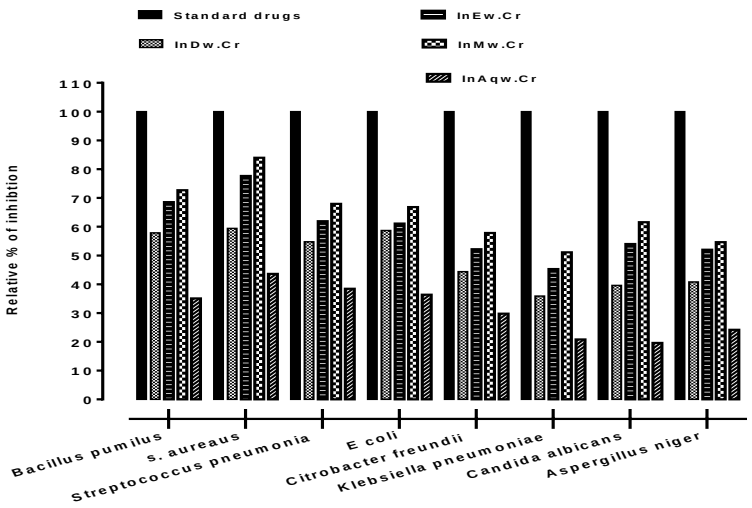


Figure 2: Relative percentage inhibition of the DCM, ethanolic, methanolic and aqueous extract of the (A) seeds and (B) whole plant of the *Ipomoea nil* in diameter (mm) against different strains of bacteria and fungi (values are expressed as mean ± SEM., n = 3).; InDs.Cr = crude extract of the seeds of the *Ipomoea nil* in DCM, InEs.Cr = crude extract of the seeds of the *Ipomoea nil* in ethanol, InMs.Cr = crude extract of the seeds of the *Ipomoea nil* in methanol, InAqs.Cr = Aqueous crude extract the seeds of the *Ipomoea nil*.; InDw.Cr = crude extract of the whole plant of the *Ipomoea nil* in DCM, InEw.Cr = crude extract of the whole plant of the *Ipomoea nil* in ethanol, InMw.Cr = crude extract of the whole plant of the *Ipomoea nil* in methanol, InAqw.Cr = Aqueous crude extract the whole plant of the *Ipomoea nil*.

4. DISCUSSION

Researchers are mainly focusing to the medicinal plants, and are trying their best to develop new natural products from medicinal plants against multidrug resistant microbial strains (Braga et al., 2005). Medicinal plants are the main source of the secondary metabolites and the pharmaceuticals. Secondary metabolites isolated from the medicinal plants have been reported to possess the antimicrobial property (Hussain et al., 2013). In the present study disc diffusion method was adopted to investigate the antimicrobial spectrum of the organic (DCM, methanolic and ethanolic) and aqueous extract of the seeds and whole plant of *Ipomoea nil* against different strains of bacteria and fungi. Our output clearly indicates that the organic extract (methanolic, ethanolic) of the seed of the *Ipomoea nil* possess the greater potential against pathogenic bacteria and fungal species as compared to the whole plant organic extract and support the view, that medicinal plants might be useful in the development of novel antimicrobial agents (Heinrich, 2001). Extensive and empirical use of the antibiotics in the management of infectious disease is the main cause of the bacterial resistance especially against *Staphylococcus aureus* (Timothy and Whitman, 2008). Methanolic extract (organic fraction) of the seeds showed significant antibacterial activity against different pathogenic species of G +ve bacteria, i.e., *Bacillus pumilus, Staphylococcus aureus,* and *Streptococcus pneumoniae*, with relative percentage of inhibition of 81.76 %, 89.88 %, and 77.10 % respectively, as compared with standard vancomycin, flucloxacillin and ceftriaxone (10 µg/disc), respectively and with MIC value of 30 µg/ml. Multidrug resistant

bacteria i.e., G –ve bacteria have limited the efficacy of antibiotics against infections (Abbanat et al., 2008). In earlier studies, researchers have found that G –ve bacteria are less susceptible to plant extracts (Kuhnt et al., 1994) but in our study, we observed that methanolic extract of the seed of the *Ipomoea nil* has good activity against *Escherichia coli,* with relative percentage of inhibition of 74.30 %, as compared with ceftriaxone (10 µg/disc), with MIC value of 90 µg/ml. Antimicrobial activity of the plant against both G +ve and G-ve bacteria and fungi, may be indicative of the presence of the broad spectrum antibiotic compounds in plant (Vaghasiya and Chanda, 2007).

The descending sequences of antimicrobial activity of various extracts of the seeds of the *Ipomoea nil* against studied microorganisms were as follow: InMs.Cr, InEs.Cr, InDs.Cr and InAqs.Cr whereas same descending sequence was also followed by the various extracts of the whole plant of the *Ipomoea nil* against same studied microorganisms but whole plant extract showed the less potent antimicrobial response as compared to seed's extract of *Ipomoea nil.*

Ipomoea nil is believed to possess the excellent antibacterial activity due to presence of tannin, alkaloids and flavonoids which have been studied (Draughon, 2004). Tannins have important role such as stable and potent antioxidants (Trease and Evans, 1983). Most of the organisms used in the research study were causative agents of diarrhea and dysentery, while *Ipomoea nil* inhibit the growth of these microbes, it can be used for the treatment of diarrhea and dysentery. Moreover, this study can be used as a tool for the isolation of pure antimicrobial from the plant and more works need to be done with the view of their use for *in-vivo* studies.

Conflict of Interests

Authors declared no competitive interests for the presented work.

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