ISSN 2231–5683 (Print)
2231–5691 (Online)
DOI: 10.5958/2231-5691.2018.00016.3

Vol. 08| Issue-02|
April- June 2018

Available online at
www.anvpublication.org

Asian Journal of Pharmaceutical Research (AJPRes.)
Home page *www.asianjpr.com*

RESEARCH ARTICLE

Antibacterial Activity of Leaves and Flowers of *Ipomoea aquatica* Forsk. (Convolvulacea)

Mital N. Manvar
Department of Pharmacognosy, Atmiya Institute of Pharmacy, Yogidham Campus, Kalawad Road, Rajkot, Gujarat, India.
*Corresponding Author E-mail: **mital_manvar@rediffmail.com**

ABSTRACT:
Man has been using herbs and plants products for combating diseases since times immemorial. Up till now, however, all antimicrobial substances from higher plants have been found either to be toxic to animals or not competitive therapeutically with the products of microbial origin, due to their low potency and narrow spectrum. Therefore, no antimicrobial compound from a higher plant has yet come into significant clinical use. Research, however, continues in the hope of finding plant antimicrobials that are effective for the systemic or topical treatment of human or agricultural infections. Extracts of leaves and flowers of *Ipomoea aquatica* were investigated for their antibacterial activity using agar cup method on the gram positive bacteria *Staphylococcus aureus*. The preliminary phytochemical screening of methanol extracts of leaves of *I. aquatica* showed the presence of flavonoids while that of the flowers showed presence of flavonoids and anthocyanins. The methanol extracts of both leaves and flowers were showed the antibacterial activity that may be due to flavonoids present in these extracts.

KEY WORDS: *Ipomoea aquatica,* Convolvulaceae, *Staphylococcus aureus,* antimicrobials, agar cup method.

INTRODUCTION:
Numerous investigations have been carried out in individual plants and the respective antimicrobial agents have been identified in a gratifying number of cases. From various studies, it is clear that the chemical structures of these agents belong to the most commonly encountered classes of higher plant secondary metabolites[1]. Up till now, however, all antimicrobial substances from higher plants have been found either to be toxic to animals or not competitive therapeutically with the products of microbial origin, due to their low potency and narrow spectrum.

Received on 20.02.2018 Accepted on 28.04.2018

Asian J. Pharm. Res. *2018; 8*(2)*: 94-98.*
DOI: 10.5958/2231-5691.2018.00016.3

Therefore, no antimicrobial compound from a higher plant has yet come into significant clinical use[1].

Research, however, continues in the hope of finding plant antimicrobials that are effective for the systemic or topical treatment of human or agricultural infections. Occurrence of infrequent variation in concentrations within species and related organisms suggest that resistance to the individual extracts, when it occurs, is due to the intrinsic properties of the species involved rather than acquired characters. For this reason, it would be more effective and much more useful if the plant extract or its active constituents can be exploited in the development of antimicrobial chemotherapeutic agents[1]. This can be considered in the line with the current search for such substances to augment or replace the antibiotics in current clinical uses which because of the spread of resistance are less useful than before. In the present study focused on antibacterial activity of plant extract and to find out possible secondary metabolite responsible for that.

Ipomoea aquatica Forsk (syn. *Ipomoea reptans* Linn.) of family Convolvulaceae; is perennial herb and distributed throughout India. According to Unani, it is carminative, lessens inflammation; useful in fever, jaundice, biliousness, bronchitis, liver complaints[2,3].

MATERIAL AND METHOD:

Plant material and preparation of extracts:

The leaves and flowers of the plant *Ipomoea aquatica* were collected from Rajkot district of Gujarat, India. The plant material was identified by Botany Dept., Kotak Science College, Rajkot and a voucher specimen (Voucher No. ARGH-10) has been retained in Department of Pharmacognosy, A. R. College of Pharmacy, Vallabh Vidyanagar, Gujarat. The collected plant materials were dried under shade and then powdered with a mechanical grinder and stored in an air-tight container, the dried powder material of both leaves and flower were extracted successively with petroleum ether, toluene, chloroform, acetone, methanol and water in a Soxhlet apparatus. The solvents were completely removed under reduced pressure and residues were obtained.

Antibacterial activity:

The antibacterial activity was carried out using agar cup method[4] on the gram positive bacteria *Staphylococcus aureus*. All the extracts petroleum ether, toluene, chloroform, acetone, methanol and water of both leaves and flowers of *I. aquatica* were subjected for this study. Different concentrations (50 mcg/ml, 100mcg/ml, 150 mcg/ml, 200mcg/ml, 250mcg/ml, 300mcg/ml and 350mcg/ml) of all the extracts were prepared and used in the study. Ciprofloxacin (5 mcg/ml) was used as standard.

Agar cup method[4]: 0.2 ml of young test culture (*Staphylococcus aureus* culture was prepared in sterile distilled water) was inoculated in melted top agar (which was previously sterilized by autoclaving) previously cooled to 50ºC. It was mixed well and poured over the nutrient agar (which was previously sterilized by autoclaving) and was allowed to solidify. The cup-borer was sterilized by dipping it in alcohol followed by flaming it. Four cups were made with the help of cup-borer, one in each quadrant, at equal distance in nutrient agar plate previously seeded with culture. Each cup was filled with definite quantity of different dilutions of extracts. The plates were incubated in refrigerator at 4-5ºC for 30 minutes so as to allow diffusion of extracts. The plates were incubated in upright position at 37ºC for 24 hours in incubator. After 24 hours the plates were observed for zone of inhibition. The zone of inhibition was measured using a ruler. The experiment was performed in triplicates for all the test samples.

RESULT AND DISCUSSION:

The extracts of both leaves and flowers of *I. aquatica* were subjected for the antibacterial study. The petroleum ether, toluene, chloroform, acetone and water extracts of both leaves and flowers were not showed antibacterial activity, while the methanol extracts of both leaves and flowers were showed the antibacterial activity. The zone of inhibition obtained at different concentration of the methanol extracts of leaves and methanol extracts of flowers of *I.aquatica* are shown in table 1. The zone of inhibition obtained of the methanol extracts of leaves of *I.aquatica* at concentrations 250mg/ml, 300mg/ml and 350mg/ml is shown in Figure 1. The zone of inhibition obtained of the methanol extracts of flowers of *I.aquatica* at concentrations 250mg/ml, 300mg/ml and 350mg/ml is shown in Figure 2. The results show that the zone of inhibition was increased with increase of the concentration of the extracts in both leaves and flowers of *I. aquatica.* The results revealed that the leaves were somewhat more active than that of the flowers of *I. aquatica.*

Table 1: Effect methanol extracts of leaves and flowers of *Ipomoea aquatica* in agar cup method.

Test sample	Concentration (mcg/ml)	Zone of Inhibition (mm±SEM) includes the diameter of disc (6 mm)
Ciprofloxacin	5	28±0.52
Methanol extract of leaves of *Ipomoea aquatica*	50	NI
	100	14±0.45
	150	16±0.64
	200	18±0.33
	250	20±0.57
	300	23±0.25
	350	26±0.53
Methanol extract of flowers of *Ipomoea aquatica*	50	NI
	100	13±0.47
	150	16±0.38
	200	18±0.54
	250	20±0.40
	300	22±0.24
	350	25±0.68

NI: No inhibition. Values are expressed as mean±SEM, (N=3).

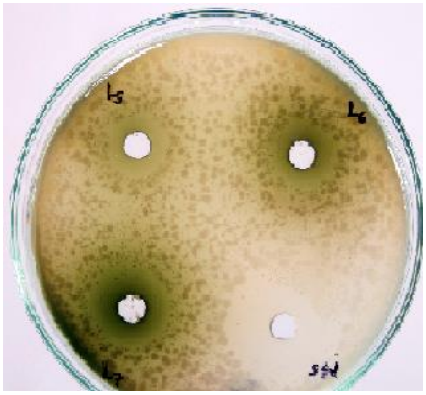

Figure 1: Zone of inhibition at different concentrations of methanol extract of leaves of *Ipomoea aquatica* (L_5:250mg/ml, L_6:300mg/ml and L_7:350mg/ml) and standard (5 mcg/ml)

Figure 2: Zone of inhibition at different concentrations of methanol extract of flowers of *Ipomoea aquatica* (F_5:250mg/ml, F_6:300mg/ml and F_7:350mg/ml) and standard (5 mcg/ml)

The results of preliminary phytochemical screening of methanol extracts of leaves and flowers of *I. aquatica* are shown in Table 2 and Table 3 respectively. The preliminary phytochemical screening of methanol extracts of leaves of *I. aquatica* showed the presence of flavonoids while that of the flowers showed presence of flavonoids and anthocyanins. Thin layer chromatography study of methanol extract of both leaves and flowers of *I. aquatica* was again confirmed presence of flavonoids [(5)]. The plant flavonoids show antibacterial activity[(6)]. Thus the flavanoids present in methanol extracts of leaves and flowers of *I. aquatica* may responsible for antibacterial activity.

Table 2: The results of preliminary phytochemical screening with the extracts obtained by successive solvent extraction of leaves of *Ipomoea aquatica*

No.	Chemical tests	Extracts					
		PE	T	C	A	M	W
1.	**Test for Alkaloids:**						
	(a) Dragendorff's test	*	*	–ve	*	–ve	–ve
	(b) Wagner's test	*	*	–ve	*	–ve	–ve
2.	**Test for Carbohydrates:**						
	(a) Molish's test	*	*	*	*	+ve	+ve
	(b) Fehling's test	*	*	*	*	+ve	+ve
3.	**Test for Saponin Glycosides:**						
	(a) Foam test	*	*	*	*	–ve	–ve
	(b) Heamolytic test	*	*	*	*	–ve	–ve
4.	**Test for Flavonoids:**						
	(a) Shinoda test	*	*	*	+ve	+ve	–ve
	(b) Lead acetate test	*	*	*	+ve	+ve	–ve
5.	**Test for Phenolic compounds and Tannins:**	*	*	*	–ve	–ve	–ve
	(a) Ferric chloride test	*	*	*	–ve	–ve	–ve
	(b) Lead acetate test						
6.	**Test for Anthocyanins:**						
	(a) With 2M	*	*	*	*	–ve	–ve
	(b) With 2M NaOH	*	*	*	*	–ve	–ve
7.	**Test for Phytosterols:**						
	(a) Liebermann's test	+ve	*	*	+ve	–ve	*
	(b) Liebermann- Burchard's test	+ve	*	*	+ve	–ve	*
8.	**Test for Fixed oils and Fats:**						
	(a) Spot test	–ve	–ve	*	*	*	*
	(b) Tincture alkana test	–ve	–ve	*	*	*	*
9.	**Test for Proteins and Amino acids:**						
	(a) Biuret test	*	*	*	*	+ve	–ve
	(b) Million's test	*	*	*	*	+ve	–ve

PE: Petroleum ether (60-80^{o}C) extract; T: Toluene extract, C: Chloroform extract; A: Acetone extract; M: Methanol extract; W: Water extract; +ve: positive; -ve: Negative; *: Not done

Table 3: The results of preliminary phytochemical screening with the extracts obtained by successive solvent extraction of flowers of *Ipomoea aquatica*

No.	Chemical tests	Extracts					
		PE	T	C	A	M	W
1.	**Test for Alkaloids:**						
	(a) Dragendorff's test	*	*	–ve	*	–ve	–ve
	(b) Wagner's test	*	*	–ve	*	–ve	–ve
2.	**Test for Carbohydrates:**						
	(a) Molish's test	*	*	*	*	+ve	+ve
	(b) Fehling's test	*	*	*	*	+ve	+ve
3.	**Test for Saponin Glycosides:**						
	(a) Foam test	*	*	*	*	–ve	–ve
	(b) Heamolytic test	*	*	*	*	–ve	–ve
4.	**Test for Flavonoids:**						
	(a) Shinoda test	*	*	*	+ve	+ve	+ve
	(b) Lead acetate test	*	*	*	+ve	+ve	+ve
5.	**Test for Phenolic compounds and Tannins:**						
	(a) Ferric chloride test	*	*	*	–ve	–ve	–ve
	(b) Lead acetate test	*	*	*	–ve	–ve	–ve
6.	**Test for Anthocyanins:**						
	(a) With 2M HCl	*	*	*	*	+ve	–ve
	(b) With 2M NaOH	*	*	*	*	+ve	–ve
7.	**Test for Phytosterols:**						
	(a) Liebermann's test	+ve	*	*	–ve	–ve	*
	(b) Liebermann- Burchard's test	+ve	*	*	–ve	–ve	*
8	**Test for Fixed oils and Fats:**						
	(a) Spot test	–ve	–ve	*	*	*	*
	(b) Tincture alkana test	–ve	–ve	*	*	*	*
9	**Test for Proteins and Amino acids:**						
	(a) Biuret test	*	*	*	*	–ve	+ve
	(b) Million's test	*	*	*	*	–ve	+ve

PE: Petroleum ether (60-80^{o}C) extract; T: Toluene extract, C: Chloroform extract; A: Acetone extract; M: Methanol extract; W: Water extract; +ve: positive; -ve: Negative; *: Not done

CONCLUSION:

The results of the present study revealed that the methanol extract of both leaves and flowers of *I.aquatica* Forsk possess antibacterial activity against *Staphylococcus aureus*. That may be possible that the antibacterial activity of the methanol extracts of both leaves and flowers of *I.aquatica* may due to presence of flavonoids. The present study provided a plate-form for further research for antibacterial study on other strains of bacteria and to identify the components responsible for this activity.

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