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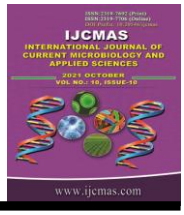

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Phyto-chemical Screening and Anti-Microbial Analysis of a Medicinal Plant: *Telosma pallida* L.

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A B S T R A C T

The work is undertaken to evaluate the preliminary phyto-chemicals, anti-microbial activity of root, stem, leaf, flower and fruit extracts. The highest anti-fungal activity was observed against *M. phaseolina* by aqueous extract of flower. The highest anti-bacterial activity was observed against *S. typhi* by methanol extract of leaf. This medicinal plant was the source of the secondary metabolites i.e., alkaloid, flavonoids, saponin etc. The outcomes of the present study specified the plant possess various potentially active secondary metabolites which help for the developing pharmaceuticals, especially antimicrobial drugs. It is the first-ever information about this plant and it will help with pharmacological studies of *Telosma pallida* L.

Introduction

Telosma pallida L., commonly known as *Pergularia pallida* L., is an undiscovered ayurvedic medicinal plant belongs to the Asclepiadaceae family. In Gujarati, this plant is known as "Radarudi" or "Varshadodi," and in Hindi, it is known as "Surkilla." The somatic chromosome number is 2n=22. This valuable medicinal plant is available and grown in India, Myanmar, Nepal, Pakistan, Thailand, Vietnam etc. The flowering season is July to September i.e. during monsoon. It is traditionally used to balance the Tridosha (vata, pitta, and kapha) energies. The leaves have an astringent flavour and a distinct odour (Kanakhara *et al.*, 2018). It has long been used to treat whooping cough, the common cold, and asthma.

Phyto-chemicals are naturally occurring compounds in medicinal plants which have defence mechanism and protect various diseases (Motaleb, 2011). In recent years natural antibiotics have been used for several infectious diseases, regarding to this, the work

on new antimicrobial agents from plants are even more essential especially in the countries like India where infectious diseases of micro-organisms are not only rapid but are also more resistant to common antibiotics (Shah *et al.*, 2014).

Materials and Methods

Plant material collection

Plant material was collected during the July to September months from Joshipura area (21.55711ºN, 70.44772ºE) of Junagadh district, Gujarat, India (Figure 1). Plant parts were then dried in shed naturally then in hot air oven at 40°C for an hour just before starting the extraction process.

Preparation of plant extract

15 g powder of leaves, flowers, fruits, stem and root extracted with four solvents (150 ml) via petroleum ether, chloroform, methanol and water based on polarity index. Then placed in a shaker at room temperature for 24 h at 200 rpm. The filtrates were concentrated by drying in a water bath at 35–40°C for methanol and at 70^0C for aqueous extract until a residues were obtained. Each of the four extracts was dissolved in DMSO (Dimethyl sulfoxide) to produce solutions of concentration 25 mg/ml.

Phyto-chemical Analysis

The obtained solvent extracts were subjected to qualitative phyto-chemical screening to detect the presence of various phytoconstituents. Methods were used for detection of alkaloid and carbohydrates test by Alfalluos *et al.,* (2017) and Edrah *et al.,* (2016); flavonoids test by Harborne, (1973) and Tiwari *et al.,* (2011); steroids and triterpenoids by Suryawanshi and Vidyasagar, (2016); test for phenolic by Shabi *et al.,* (2014); test for glycosides by Kumar *et al.,* (2013) and Gul *et al.,* (2017); test for tannins and coumarins by Cai *et al.,* (2011), Ismail *et al.,* (2017), Mondal *et al.,* (2017) and Nandagoapalan *et al.,* (2016); test for saponins by Auwal *et al.,* (2014).

Anti-microbial activity

Test micro-organisms

Test organisms were collected from the culture collection of the Institute of Microbial Technology (IMTECH), Chandigarh. These included *Bacillus subtilis* (MTCC NO. 8114), *Escherichia coli* (MTCC NO. 443), *Staphylococcus aureus* (MTCC NO. 3160), *Salmonella typhi* (MTCC NO. 3216), *Klebsiella pneumoniae (MTCC NO. 109), Fusarium oxysporum* (MTCC NO. 6654), *Candida albicans* (MTCC NO. 227). *Aspergillus niger, Aspergillus flavus and Macrophomina phaseolina* culture were taken from microbial cell, food testing laboratory, J. A. U., Junagadh. Streptomycin and ampicillin were taken as standard antibiotics for comparing the antibacterial activity. Hexa antimyco-01 was used as standard for anti-fungal activity.

Agar-well Diffusion Method

Hundred µl of bacterial or fungi culture was pipetted in the centre of sterile petri dish of Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates. It was spread by using L shape spreader. Four wells were made using a sterile cork borer. Hundred µl of four plant solvent extracts were added into the wells. Control experiments comprising inoculums with DMSO as negative control and standards. The plates were incubated at 37°C for 24 hr for bacterial pathogens and 28°C for 48 hr fungal pathogens. The diameter of the inhibition zone (mm) was measured by himedia antibiotic zone scale and the activity Index (AI) was calculated by the established formula (Sharma

and Kumar, 2017). Activity Index (AI) = Inhibition zone of the sample/ Inhibition zone of standard. Duplicates were maintained and the experiment was repeated twice, average values were recorded.

Results and Discussion

Phyto-chemical Screening

The preliminary phyto-chemical screening of crude extracts of various solvents showed the presence of secondary metabolites were tabulated in the (Table 1).

Alkaloids have potentials to show anti-microbial activity (Plumb *et al.,* 1999). Alkaloids were widely distributed in all parts (Table 1). All the three tests of alkaloids were present in chloroform extracts of all parts except Hager's test in stem, flower and fruits. Flavonoids were remain presents in all parts of plant in either test. Flavonoids and terpenoids are known to possess anti-microbial, anti-viral, anti-allergenic, anti-inflammatory and immune-modulatory properties (Rabi and Bishayee, 2009). Coumarin was not observed in any of the extracts of fruit. Findings from the study was similar to those obtained in methanol and water extract of leaf and stem by Sharma *et al.,* (2014) and water, methanol, chloroform extract of leaf by Kanakhara *et al.,* (2018).

Anti-fungal activities

M. phaseolina is one of the most destructive soil-borne fungus. Under favourable environmental conditions, the fungus causes charcoal rot and damping off in more than 500 plant species belonging to about 100 angiospermic families (Ijaz *et al.,* 2013). Among all selected fungal strains, aqueous extract of flower had the highest effect on *M. phaseolina*, with a zone of inhibition 16 mm and activity index.

In general methanol extract of leaf, flower, stem, fruit; ether extract of flower and fruit as well as chloroform extract of flower do not have any effect against selected fungal strains (Table 3) (Fig. 1).

Maximum inhibitory zone of standard antibiotic nystatin 23 mm was observed. *A. niger* causes black mold disease on specific fruits and vegetables, instigate allergic problems in human due to its ability to produce certain mycotoxins which are hepatocarcinogenic, nephrogenic immunological in nature (Sharma, 2012).

Maximum inhibitory zone of standard antibiotic nystatin 15 mm was observed. *Fusarium oxysporum* is a species complex comprising ubiquitous soil borne plant pathogens with more than 150 host-specific forms (Asha *et al.,* 2011). Maximum inhibitory zone of standard antibiotic nystatin 15 mm was observed. *C. albicans* is commonly used as a model organism for fungal pathogens.

Aspergillus flavus is mostly found in soil as saprophytes, but it has a broad host range as an opportunistic pathogen (Tilak *et al.,* 2016).

It was find out that methanol and aqueous extract of root showed maximum zone of inhibition of 12 mm and 12.5 mm, respectively against *Aspergillus flavus*. Likewise, chloroform extract of leaf, root and fruit showed 10.0 mm, 13.0 mm and 12.0 mm zone of inhibition, respectively. Maximum anti-fungal activity of standard ketoconazole was 24 mm (Table 2).

Anti-bacterial activities

In the anti-bacterial activity, among all five bacteria, *S. typhi* exposed more sensitivity to methanol extract of leaf and had the maximum

zone of inhibition 12 mm, whereas water extract of flower showed minimum zone of inhibition against *S. typhi* (8.5 mm). *S. typhi (gram negative)* are bacteria that infect the intestinal tract and the blood. The disease is referred to as typhoid fever (Gehlot and Bohra, 2000). *Bacillus subtilis* is model system for gram-positive organisms. Chloroform extract of stem had valuable results against *E. coli* (gram negative) with inhibition zone 11 mm followed by aqueous extract of root (10.5 mm). It can cause serious food poisoning, urinary tract infections, diarrhoea, serious illness or death (Kurhekar *et al.,* 2013). Only ether extract of stem show anti-bacterial activity against *S. aureus* with 10 mm of zone of inhibition. *S. aureus* (gram positive) causes serious infections of the skin, soft tissues, bone, lung, heart, brain or blood (Desalegn,2014).

Water extract of fruit showed maximum anti-bacterial activity against *K. pneumoniae* with 11 mm of zone of inhibition. *K. pneumoniae* (gram negative) is the t opportunistic pathogen that can cause pneumonia and urinary tract infections (Wu *et al.*, 2012). Among the five tested bacteria, the growth of *S. typhi* was more effected by the plant extracts followed by *B. subtilis* and *E. coli* (Table 5) (Fig. 2). Zone of inhibition of standard streptomycin and ampicillin was reported (Table 4).

It was observed that *M. phaseolina* and *S. typhi* were most susceptible among selected microbial strains. In the current study, Staphylococcus aureus was found to be most resistant. Similar results were reported by Reddy (2009) that in anti-microbial activity of *Datura stramonium* L. and *Tylophora indica* L. against *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus niger* and *Fusarium* species.

Table.1 Inhibition zone (mm) of standard used for anti-fungal activity

Standards	Clotrimazole (CC)	Fluconazole (FLC)	Itraconazole (IT)	Ketoconazole (KT)	Nystatin (NS)	Amphotericin-B (AP)
A. niger	13	-	10	14	23	-
F. oxysporum	-	-	-	-	15	-
A. flavus	15	-	15	24	14	-
C. albicans	10	-	-	-	15	12
M. phaseolina	-	-	10	-	-	-

Table.2 Inhibition zone (mm) of standard used for anti-bacterial activity

Standard	Streptomycin	Ampicillin
B. subtilus	22	-
S. typhi	24	-
E. coli	27	-
S. aureus	24	-
K. pneumoniae	27	-

Table.3 Preliminary Phyto-chemical screening of *Telosma pallid*

No.	Phyto-chemical	Tests/Parts	Root				Stem				Leaf				Flower				Fruit			
		Extracts	E	C	M	W	E	C	M	W	E	C	M	W	E	C	M	W	E	C	M	W
1.	Alkaloid	Wagner's test	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
		Mayer's test	-	+	+	+	-	+	+	-	+	+	-	-	-	+	-	-	+	+	-	-
		Hager's test	-	+	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-	-	-
2.	Flavonoids	Alkaline reagent test	-	+	-	-	+	+	-	+	+	+	-	-	+	+	-	-	+	+	-	-
		Lead acetate test	-	-	+	+	-	-	+	+	+	+	+	+	-	-	+	+	-	-	+	+
3.	Steroids & TT	Salkowski test	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-	+	+	-	-
4.	Saponin	Froth test	-	+	-	-	-	+	-	-	-	+	-	+	-	+	-	-	-	+	-	+
5.	Glycosides	Killer-Killer test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Liberman's test	+	+	+	-	-	+	+	-	+	+	+	-	+	+	+	-	+	+	+	+
6.	Carbohydrates	Benedict's test	-	-	-	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
7.	Tannin	Dichromate test	-	-	-	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
		Ferric chloride test	-	-	-	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
		Modified prussian blue test	-	-	-	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
8.	Phenol	$FeCl_3$ test	-	-	+	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	-
9.	Coumarin	NaoH test	-	+	+	-	+	+	-	-	-	+	-	-	+	+	-	-	-	-	-	-
		Open-loop Close-loop test	-	+	+	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	-	-

Table.4 Anti-fungal activity of *Telosma pallida* against selected fungal strains

Anti-fungal Activity																	
No.		**Root**				**Stem**			**Leaf**			**Flower**			**Fruit**		
	Extract	**E**	**C**	**M**	**W**	**E**	**C**	**W**	**E**	**C**	**W**	**E**	**C**	**W**	**E**	**C**	**W**
1.	*A. niger*	-	-	-	-	-	-	-	-	-	-	11.5 (0.50)	11.0 (0.47)	-	-	-	-
2.	*F. oxysporum*	10.5 (0.70)	-	SL	-	-	-	-	-	-	-	-	-	-	-	-	10.0 (0.60)
3.	*A. flavus*	11.0 (0.45)	10.0 (0.41)	-	-	-	-	-	-	12.0 (0.50)	12.5 (0.52)	-	-	11.0 (0.45)	-	-	11.0 (0.45)
4.	*C. albicans*	9.0 (0.60)	10.5 (0.70)	10.0 (0.66)	-	-	-	-	-	-	-	-	-	-	-	-	-
5.	*M. phaseolina*	15.0 (1.50)	14.0 (1.40)	-	-	-	16.0 (1.60)	15.0 (1.50)	13.0 (1.30)	-	-	-	15.5 (1.55)	11.0 (1.10)	-	12.0 (1.20)	14.5 (1.45)

The data are expressed as the mean in millimeter and recorded after 24 hours.
- = No activity,SL= SLight zone, E= Petroleum ether, C= Chloroform, M=Methanol, W=Water
Figures in parenthesis are activity index (AI).

Table.5 Anti-bacterial activity of *Telosma pallida* against selected bacterial strains

Anti-bacterial Activity																				
No.		**Root**				**Stem**				**Leaf**				**Flower**				**Fruit**			
	Extract	**E**	**C**	**M**	**W**	**E**	**C**	**M**	**W**	**E**	**C**	**M**	**W**	**E**	**C**	**M**	**W**	**E**	**C**	**M**	**W**
1.	*B. subtilis*	-	-	SL	8.5 (0.38)	-	-	-	SL	8.0 (0.36)	SL	SL	8.0 (0.36)	8.0 (0.36)	-	11.0 (0.50)	10.5 (0.47)	-	10.5 (0.47)	SL	11.0 (0.50)
2.	*S. typhi*	8.0 (0.33)	SL	10.0 (0.41)	11.0 (0.46)	-	10.0 (0.41)	8.0 (0.33)	-	-	10.0 (0.41)	12.0 (0.50)	11.5 (0.47)	SL	-	10.0 (0.41)	8.5 (0.35)	-	-	-	-
3.	*E. coli*	-	10.0 (0.37)	SL	10.5 (0.38)	10.0 (0.37)	11.0 (0.40)	10.0 (0.37)	-	-	-	-	-	-	-	-	-	-	-	-	9.0 (0.33)
4.	*S. aureus*	-	-	-	-	10.0 (0.43)	-	SL	-	-	-	-	-	-	-	-	-	-	-	-	-
5.	*K. pneumoniae*	-	SL	-	8.0 (0.29)	10.0 (0.37)	SL	-	-	-	-	-	-	-	-	-	-	-	10.0 (0.37)	-	11.0 (0.40)

The data are expressed as the mean in millimeter and recorded after 48 hours.
- = No activity, SL= SLight zone, E= Petroleum ether, C= Chloroform, M= Methanol, W= Water
Figures in parenthesis are activity index (AI).

This medicinal plant was the source of the secondary metabolites i.e., alkaloid, flavonoids, steroids and triterpanoids, saponin, glycosides, carbohydrates, tannin, phenol, coumarin. The higher anti-fungal activity was observed in aqueous extract of flower against *M. phaseolina*. The highest anti-bacterial activity was observed against *S. typhi* by methanol extract of leaf. It is the first-ever information about this plant and it will help with pharmacological studies of *Telosma pallida* L.

References

Alfalluos, K. A., Alnade, H. S., Kollab, W. A., Alafid, F. and Edrah, S. M. 2017. Qualitative and quantitative phyto-chemical analysis and antimicrobial activity of "Retama" extract grown in zliten libya. *International J. Medical Science and Clinical Inventions*, 4(4):2861-2866.

Asha, B. B., Nayaka, S., Udayashankar, A. C., Srinivas, C. and Niranjana, S. R. 2011. Selection of effective bio-antagonistic bacteria for biological control of tomato wilt caused by *Fusarium oxysporum*. *Bio Scan Research*, 6:239–244.

Auwal, M. S., Saka, S., Mairiga, I. A., Sanda, K. A., Shuaibu, A. and Ibrahim, A. 2014. Preliminary phyto-chemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (*Thorn mimosa*). *Veterinary Research Forum*, 5(2): 95-100.

Cai, L. Y., Shi, F. X. and Gao, X. 2011. Preliminary phyto-chemical analysis of *Acanthopanan trifoliatus* (L.) Merr. *J. Medicinal Plants Research*, 5(17):4059-4064.

Canini, A., Alesiani, D., Arcangelo, G. and Tagliatesta, P. 2007. Gas chromatography–mass spectrometry analysis of phenolic compounds from *Carica papaya* L. leaf. *J. Food Composition and Analysis*, 20:584–590.

Desalegn, A. 2014. Anti-microbial activity of medicinal plant extracts and their synergistic effect on some selected pathogens. *American J. Ethnomedicine*, 1(1):18-29.

Edrah, S. M., Aljenkawi, A., Omeman, A. and Alafid, F. 2016. Qualitative and quantitive analysis of phyto-chemicals of various extract for *Ephedra altissima* from Libya. *J. Medicinal Plants Studies*, 4(3):119-121.

Gehlot, D. and Bohra, A. 2000. Antibacterial effect of some leaf extracts on *Salmonella typhi*. *Indian J. Medical Sciences*, 54(3):102-105.

Gul, R., Jan, S. U., Faridullah, S., Sherani, S. and Jahan, N. 2017. Preliminary phyto-chemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from ephedra intermedia indigenous to balochistan. *The Scientific World J.*, 1-7.

Harborne, J. B., 1973. Phyto-chemical methods. London: Chapman and Hall, 52-114.

Ijaz, S., Sadaqat, H. A. and Khan, M. N. 2013. A review of the impact of charcoal rot (*Macrophomina phaseolina*) on sunflower. *Journal of Agricultural Science*, 151:222-227.

Ismail, M., Haq, N. U., Ali, S., Ali, I., Shaheen, R., Murtaza, G. and Abdurraza, Q. 2017. Qualitative analysis for phyto-chemicals of selected medicinal plants from gilgit-baltistan, Pakistan. *Asian J. Chemistry*; 29(9):1929-1932.

Kanakhara, R. D., Rudrappa, H. C., Shukla, V. J. and Acharya, R. A detailed pharmacognostical and phyto-chemical profile on stem of *Telosma pallida* (L.) Kurz.: An extrapharmacopoeial drug. International J. Research in Ayurveda and Pharmacy, 2018, 9(1):36-43.

Kumar, R. S., Venkateshwar, C., Samuel, G. and Rao, S. G. 2013. Phyto-chemical screening of some compounds from plant leaf extracts of *Holoptelea integrifolia* (Planch.) and *Celestrus emarginata* (Grah.) used by gondu tribes at adilabad district, Andhrapradesh, India. *International J. Engineering Science Invention*, 2(8):65-70.

Kurhekar, J., V. and Bodhankar, M. 2013. *In-vitro* anti-bacterial activity of few medicinal plants against *Escherichia coli*. *Research J. Chemistry and Environment*, 17(3):49-52.

Mondal, S., Hossain, I. and Islam, M. N. 2017. Phyto-chemical screening of ethanolic extract of leaves and stems of *Cucubita pepo* Linn. *International J. Chemistry Studies*, 1(2): 32-34.

Motaleb, M. A. 2011. Selected medicinal plants of Chittagong hill tracts. International union of conservation of nature and natural resources, 1–3.

Nandagoapalan, V., Doss, A. and Marimuthu, C. 2016. Phyto-chemical analysis of some traditional medicinal plants. *Bioscience Discovery*, 7(1):17-20.

Plumb, G. W., Price, K. R. and Williamson, G. 1999. Antioxidant properties of flavonol glycosides from green beans. *Redox Report*, 4(3):123–127.

Rabi, T. and Bishayee, A. 2009. Terpenoids and breast cancer chemoprevention. *Breast Cancer Research and Treatment*, 115:223-239.

Reddy, U. B. 2009. Antimicrobial activity of *Datura stramonium* L. and *Tylophora indica* Merr. *Pharmacologyonline*, 1: 1293-1300.

Shabi, R. R., Kumari, V. and Chitarasu. 2014. Qualitative phyto-chemical characterization of thorn extracts of *Canthium parviflorum* LAM. *International Research J. Pharmaceutical and Applied Sciences*, 4(5):13-17.

Shah, P., Modi, H. A., Shukla, M. D. and Lahiri, S. K. 2014. Preliminary phyto-chemical analysis and antibacterial activity of *Ganoderma lucidum* collected from dang district of Gujarat, India. *International J. Current Microbiology Applied Sciences*, 3(3): 246–255.

Sharma, J. and Kumar, P. 2017. Comparative study of antimicrobial activity and phyto-chemical screening of serial extracts from leaves and fruit of *Aegle marmelos* and *Carica papaya*. *International J. Pharmacy and Pharmaceutical Sciences*, 9(12):119-123.

Sharma, P., Sharma, G. N., Shrivastava, B. and Jadhav, H. R. 2014. Evaluation of free radical scavenging potential of *Telosma pallida*. *J. Harmonized Research in Pharmacy*, 3(4):125-133.

Sharma, R. 2012. Pathogenecity of *Aspergillus niger* in plants. *Centre for Informattion Bio Technology J. Microbiology*, 1: 47-51.

Suryawanshi, P. and Vidyasagar, G. M. 2016. Phyto-chemical screening for secondary metabolites of *Opuntia dillenii* Haw. *J. Medicinal Plants Studies*, 4(5):39-43.

Tilak, R., Kumar, P., Rathee, R. and Dubey, K. K. 2016. Screening of some medicinal plants for their anti-microbial activities. *International J. Pharmacy and Pharmaceutical Sciences*, 8(5):202-206.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and Kaur, H. 2011. Phyto-chemical screening and extraction: A review. *Internationale Pharmaceutica Sciencia*, 1(1):98-106.

Wu, H. S., Wang, F. D., Tseng, C. P., Wu, T. H., Lin, Y. T. and Fung, C. P. 2012. Characteristics of healthcare associated and community acquired *Klebsiella pneumoniae* bacteremia in Taiwan. *J. Infection*, 64:162-168.

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