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Phytochemical and Antibacterial Potential of *Thevetia neriifolia* Juss

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ABSTRACT

Thevetia neriifolia Juss .belonging to family Apocynaceae, an important medicinal plant was subjected to phytochemical screening and antibacterial potentials. The antibacterial activity for *Thevetia neriifolia* leaves and flowers was performed by well diffusion assay against human pathogen bacteria strains such as *Bacillus subtilis, Bacillus cereus, Bacillus megaterium, Bacillus coagulans,* and *Bacillus licheniformis,*. The present investigation deals with an important medicinal plant *Thevetia neriifolia*, leaves and flowers extract with aqueous, ethanol, chloroform and petroleum ether were performed for antibacterial potential. Results of the present investigation revealed that leaves and flowers extract can be used as a good source of antibacterial agent and as a natural antioxidant justified the folkloric use of this medicinal plant. Further pharmacological and phytochemical investigation may useful in to generate new effective antibacterial drugs in future. Leaves extract of *Thevetia neriifolia* was showed better inhibitory effect on the test pathogen than flower. Compared to the other extract the chloroform extract was showed at 50% concentration by (26.78 ± 1.38) marked inhibitory activity on *Bacillus megaterium* followed *Bacillus subtilis, Bacillus cereus, Bacillus coagulans,* and *Bacillus licheniformis.* Phytochemical constituent is also detected in the above-said plant.

INTRODUCTION

Medicinal plants as a group comprise approximately 8000 species and around 50% of all the higher flowering plant species of India. Millions of rural households use medicinal plants in a self-help mode. Half million practitioners of the Indian system of medicine in the oral codified streams use medicinal plants in preventive, promotive and curative application. There are estimated to be over 7800 manufacturing units in India, in recent years, the growing demand for herbal product has led to a quantum jump in volume of plant metabolite previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents.

Nearly 80 % of the world population some plant accessions collected from all part depends upon traditional systems of health care. (Hutchings., 1996). In recent years secondary metabolites and phytochemicals, previously with unknown pharmacological activities, have been extensively investigated as a source of medical agents (Krishnaraju. 2006).

Thevetia neriifolia is commonly known as Yellow Oleander. Scientifically it is also known as *Thevetia peruviana*. This plant contains different glycosides in every part of it esp. cardiac glycosides (Odhiambo*et al*., 2012. Jen *et al.*, 2015). Heart failure today's era has become common cause of death. In spite of having such famous medicines and techniques one cannot control over it and the morality, rate is same from the past several decades. (Charfiet *al*., 2015)

Thevetia neriifolia is found to be most toxic plant among all and it has been noticed that a small does if given to human being may cause fatal death. This plant contains cardiac glycosides in it named the vetian A and B and other glycosides too (Lozano *et al.*,2015) . Cardiac glycosides have been used for centuries as therapeutic agent. Compounds containing steroids nucleus having unsaturated lactones at C – 17 position and one or may be more residues at C – 3 position too which are found in most of the plants and toad species which acts as venom and toxins that serve as protection against any predator. Glycoside in this plant also contains digoxin and its related components which are a cardiac glycoside and used in the treatment of several cardiac diseases.(Steele *et al* .,2011) Bamigboye Taiwo *et al*.,2015 , Hwang *et al.*,2015, Divekar ., *et al.*,2015 , Hasan *et al.*, 2015).

Thevetia neriifolia, Juss belonging to family Apocynaceae was reported to have many curative effects against skin infections , addition to the healing potential towards various conditions such as edema, insomnia, hemorrhoids, malaria snake bites, etc,(Nellis.,1997)

The plant is useful in urethral discharges, repelling worms, valuable against skin disorders, leucoderma, wounds and piles and is astringent to bowels. In Guiana, the seeds are used as a purgative in rheumatism and dropsy, it is also considered as a good alexiteriby. Studies conducted various investigators revealed the anti-diarrheal, cytotoxic and insecticidal activities of leaves, seeds, stem and roots in addition to antimicrobial activity against some common pathogenic bacteria and fungi.(Hassan *et al.*, 2011, Hammuel *et al.*,2011 , Mollah *et al.*,2007) . The present study was planned to investigate phytochemical and antibacterial potential of *Thevetia neriifolia.*

MATERIALS AND METHODS

Collection of plant materials

Plant material was collected from Herbal Garden; STET Women's college, Sundarakkottai, Thiruvarurdistrict, Tamilnadu. The collected plant materials were chopped into small pieces. Fresh twigs were washed thoroughly in running tap water to remove adhering dust particles and other contaminants, and dried in shade for 2 to 3 weeks at room temperature. Dried leaves and flowers were powdered finely in a homogenizer and kept in airtight containers till further assays were done. Accurately weighed sample (30 g) was extracted successively with 300 ml of each of aqueous, ethanol, petroleum ether (PE), chloroform (CH), in a Soxhlet extractor for 12 – 18 hrs. The solvent was evaporated and the concentrated extracts were kept at 4℃ for further studies.

Preparation of plant extract and solvent

The shaded dried plant material was pulverized into coarse powder and extracted in soxhlet apparatus using aqueous, ethanol, chloroform and petroleum ether extract were also prepared. The collected aqueous ,ethanol, chloroform and petroleum ether extract were concentrated under vacuum [50^0c] dried and prepared at different concentration 25%, 50%, 75% and 100% against test pathogens. Phytochemical screening was done by saponins, alkaloids, flavonoids, tannins, sterols, phenols and carbohydrate standard methods (Trease., 1985)Leaves and

flower sample of 10 g was immersed in 100 ml of distilled water. Mixed and allow soaking for 24 hrs. Then the mixer was filtered through Whatman No.4 filter paper to get pure extract.

Antimicrobial activity

Well diffusion technique was performed by (Bauer et al., 1996)

The bacterial culture used for well diffusion method one well of 5mm size made in the agar plates with the help of serial cork bork under expected condition in laminar air flow chamber. The wells were loaded with 0.5ml of the leaves and flower extract by the help of micropipette. The bacterial plates were incubation at 37^{o}c for 24 hrs.

The plates were observed after 24 hrs for clearing zone around the well. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well, the zone of diameter was measured in 5 mm. Triplicates were maintained and the experiment was repeated thrice and the average values were recorded for antibacterial.

RESULTS AND DISCUSSION

In this investigation, the active phyto-components of *Thevetia neriifolia* was studied and further the antibacterial activity of the plant extracts was assayed *invitro* by agar well diffusion method, against five gram-positive species (Table – 1).

Successive solvent extraction was chloroform (26.78 ± 1.38) on *Bacillus megaterium.* followed by (25.09 ± 0.67) on *Bacillus megaterium* and the remaining solvent followed by (24.67 ± 1.15) on *Bacillus subtilis* and followed by petroleum ether (19.00 ± 1.00) on *Bacillus coagulans* are showed little performance on the used pathogen then compared to aqueous , ethanol and petroleum ether(Table – 3)

Compared to all the extract, chloroform was given better performance for the leaves of *Thevetia neriifolia* at 50% level. In over all aspects, the pathogens are *Bacillus megaterium, Bacillus subtilis, Bacillus coagulans, Bacillus cereus* and *Bacillus licheniformis* are controlled effectively.

In our study *Thevetia neriifolia* saponins, alkaloids, flavonoids, tannins, sterols, phenols and carbohydrate are also present (Table – 2). Pragati *et al.*,(2012) revealed the presence of alkaloids, glycosides, phenolic compounds, tannins, oils and gums in methanol extracts of fresh *Thevetia* flowers. Different active compounds were used as insecticides, fungicides,

rodenticides and bactericides, clearly indicate the presence of diverse secondary metabolites in various plant parts.

In our reports similar to Atul Kumar Ganguar (2013) preliminary phytochemical screening of extracts revealed the presence of the bioactive compounds, such as alkaloids, flavonoids, anthraquinones, phenolic compounds, saponins steroids and tannins.

Petroleum ether, Benzene, chloroform, ethyl acetate, and ethanol and water extracts of drugs showed significant antibacterial activity against the human pathogens. Petroleum ether and ethanol exhibited maximum activity against the tested human pathogen, while extracts ethyl acetate and water extract showed minimum activity against some pathogen.

The ethanolic extract of *Thevetia neriifolia* leaves were showed moderate inhibitory activity against the test pathogen. The petroleum ether extract *Thevetia neriifolia* flower were performed moderate inhibitory activity on the test pathogen at 100% level.

Kaareru*et al*., (2012) prepared a skin care herbal lotion using *Thevetia* seed oil against bacteria Bacillus *cereus* and *Bacillus subtilis*. Other therapeutic uses like anti-hemorrhoidal, antiarthritic, antirheumatic and insecticidal properties of *T. Peruviana* leaf, stem and roots were also reported. Flavanone and flavonol glycosides from the leaves showed HIV- I integrase inhibitory activities, proved that the plant is a vast repository of many powerful drugs.

TABLE – 1 Morphological and biochemical characteristics

Sr No	Morphological and biochemical characteristics	*Bacillus subtilis*	*Bacillus cereus*	*Bacillus coagulans*	*Bacillus megatrium*	*Bacillus licheniformis*
1	Gram's staining	+	+	+	+	+
2	Motility	Motile	Motile	Motile	Motile	Motile
3	Shape	Rod	Rod	rod	rod	Rod
4	Indole Test	_	_	_	_	_
5	Methyl Red Test	_	+	+	_	+
6	Voges Proskauer Test	+	+	+	+	+
7	Citrate Test	+	+	+	+	+
8	Urease Test	_	_	_	_	_
9	Nitrate	_	_	_	+	_
10	Catalase Test	+	+	+	+	+
11	Oxidase Test	_	_	_	_	+

'+' positive

'_' Negative

TABLE -2 Preliminary phytochemical and screening of *Thevetia neriifolia*

Plant	Saponins	Alkaloids	Flavonoids	Tannins	Sterols	Phenols	Carbohydrate
Thevetia neriifolia	+	+	_	_	_	+	+

TABLE -3 Antibacterial potential of chloroform extracts of Thevetia neriifolia leaves

S. NO	Organisms	zone of inhibition(mm)			
		25 %	50%	75%	100%
1	*Bacillus subtilis*	19.00±1.00	20.01±0.21	17.10±0.36	21.09±0.09
2	*Bacillus cereus*	9.33± 0.58	10.43±0.64	11.40±0.12	10.98±0.12
3	*Bacillus coagulans*	18.67± 1.15	15.67±0.12	18.09±0.90	16.23±0.98
4	*Bacillus megatrium*	25.33± 0.58	26.78±1.38	21.90±0.23	20.90±0.76
5	*Bacillus licheniformis*	22.21±1.17	21.40±1.23	20.17±1.09	19.00±0.09

Values are triplicate and represented as mean ± standard deviation

CONCLUSION

The antibacterial activity for *Thevetia neriifolia* leaves and flowers was performed by well diffusion assay against human pathogen of bacteria strains such as *Bacillus subtilis, Bacillus cereus, Bacillus megaterium, Bacillus coagulans, and Bacillus lichniformis.* The present investigation deals with an important of medicinal plant *Thevetia neriifolia* , aqueous ,ethanol, chloroform and petroleum ether extracts of *Thevetia neriifolia* leaves and flowers showed enormous antibacterial potential. So, the leaves extracts can be used as a biologically safer and cheaper alternative to the high-cost pharmaceutical remedies. Further pharmacological and standardization of the herbal drugs may useful in to generate new effective antibacterial drugs in future.

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