Phytochemical screening and In-vitro antimicrobial activity of common weeds

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Abstract

The phytochemical such as alkaloids, flavinoids, steroids and phenolic compounds are of great importance towards the antimicrobial activity. These compounds can be extracted from various parts of the plants such as roots, stem leaves for curing bronchitis, cholera, dysentery, fever and diarrhea. This study is an attempt to extract the phytochemicals from the dry leaves of *Parthenium, Datura starmonium* and *Calotropis gigantea*. These are the common available weeds in India and belong to family *Asteraceae*, *Solanaceae* and *Apocynaceae* respectively. Aqueous, methanol and n-hexane extracts were used to check the presence of phytochemicals and also the efficacy of antibacterial effect on nine selected bacterial species and antifungal activity on eight fungi procured from the Genohelix lab. In conclusion *Datura starmonium* and *Calotropis-gigantea* showed the presence of alkaloids and phenolic compounds with aqueous and methanolic extracts and n-hexane extract of all the test plants has not shown the presence of any of phytochemical component. Antibacterial activity of *Datura starmonium* was highest with the zone inhibition of 18 mm with *Staphylococcus citreus* in comparision with the standard antibiotic pencillin, however, antifungal activity was not much effective.

Keywords: *Datura starmonium*, *Lantana camara*, *Calotropis*, antifungal activity and antibacterial activity.

Introduction

Ayurveda is the very old medicinal form developed 5000 years ago which deals with both the physical and spiritual health. Ayurveda give importance to herbal medicine and treatments [1]. Many herbal medicines are used like cardamom and cinnamon [2]. In growing countries microorganism are known to cause fundamental diseases**,** offering a serious public health issue in a noteworthy division of the population and was not covered by either official or private health care system [3].

The phytochemicals extracted from plants are of great importance towards antimicrobial activity these are in general secondary metabolites having therapeutic effect on various diseases. The metabolites like steroids, flavonoids, fatty acids, alkaloids, phenolic compounds and tannin are having capability to produce a physiological action on subject. These compounds can be attracted from various parts of the plants such as roots, stem leaves for curing bronchitis, cholera, dysentery, fever and diarrhea [4].

Parthenium, Datura starmonium and *Calotropis- gigantea* are the common available weeds in India and belong to family *Asteraceae, Solanaceae* and *Apocynaceae* respectively.

Parthenium is a rich source of volatile oils, flavonoids, terpenoids, phenolic derivatives and amino acid. The extract of this shows treatment against analgesic, antipyretic and-inflammatory activities [28].Very few studies revealed that this weed can also be used for the useful purposes like a substrate for biosurfactant production [2].

Datura is well known weed for its pharmological actions. The extract of this shows treatment of nervous system, skin infections, dental, infections, toothache and respiratory disorders. However this genus is also used to understand antimicrobial or antibacterial activity and hybridity [29]

Lantana camara is a popular medicine used as anti-plasmodic, carminative and antimetic. The extract of this shows treatment against cold, bronchitis, asthma and cough. It is also used as hepatotoxic activities, antifungal, antitumor and analgesic [30]

Calotropis proved to be traditional medicine for lupus, asthma, leprosy, rheumatism, syphilis and eczema (Wealth India 1992), further the properties of this plant extract reported as anticoagulant and antimicrobial activity also [36]. Understanding the background of the medicinal importance

of these weeds in the present study attempts to check the efficacy of these plant extracts towards pathogenic bacteria and fungi [31]

MATERIALS AND METHODS

The test bacterial species *Bacillus aureus, Staphylococcus aureus, Serratia, Staphylococcus citreus, Bacillus polymyxa, Klebsiella spp., Proteus mirabilis, Salmonella typhi ,Pseudomonas aeruginosa* are procured from Genohelix Biolabs Bangaluru India.Also the fungi used in this study are *Candida albicans, Candida parapsilosis, Cryptococcus, Aspergillus oryzae, Aspergillus flavus, Aspergillus niger, Trichophyton mentageophytes, Penicillium, Trichoderma, and Trichophyton rubrum* procured from the same lab**.** These microbes were maintained on nutrient agar and Sabouraud Dextrose agar slants [1], respectively at 4ºC throughout the study and use as stock culture.

The study plants *Parathenium, Datura starmonium* and *Calotropis-gigantea* were collected from outskirts of Bellary and *Lantana camara* were collected from outskirts of Bangalore.

Aqueous extract

The leaves of the *Parathenium, Datura starmonium, Lantana camara* and *Calotropis was* brought to the laboratory washed under running tap water and dried in hot air oven at 60 degree Celsius. The dried leaves were powdered using blender and kept for further use.5gm of each powder sample was dissolved in 50ml of distilled water under sterilized condition. The setup is placed in rotatory shaker for 48 to 72 hr and centrifuge for 30 minutes at 5000rpm maintaining a temperature of 40℃.The precipitate was discarded and the supernatant was used for further experiments.

Soxhlet extract

The soxhlet extraction was carried out for all the samples by filling the thimble with 5gm of dried leaf powder respectively using 50 ml of methanol and n-hexane solvents [12, 13]. The collected extract is stored at 4℃ for further use.

Qualitative estimation of phytochemicals

The following tests were carried out to determine the phytochemical presentation of three extract

Alkaloids[14]**:**The total volume of 500microlitre sample contains solvent was allowed to evaporate by heating .**Mayer's test** [1, 15]: This test was carried out by taking 1ml of sample and adding 2 to 3 drops of Mayer's reagent in the walls of the test tube. The presence of alkaloids was confirmed by the appearance of white precipitate in the given sample. **Wagner's test** [15]: 2 to 3 drops of wagner's reagent along with 1ml of sample was added into the test tube. The presence of alkaloids was confirmed by the appearance of reddish brown precipitate in the given sample solution. **Herger's test** [14]: In the test tube 2ml of sample contains 1ml of Herger's reagent. The presence of alkaloids was confirmed by the appearance of yellow precipitate in the given sample. **Dragendroff test** [14]: 2ml of Dragendroff reagent with 1ml of sample was added in the test tube. The presence of alkaloids was confirmed by the appearance of yellow precipitate in the given sample. **Carbohydrate** [14]: The solvent was evaporated by heating 500microlitre of sample and then the dried extract was dissolved in 1ml of distilled water and stored for further use. **Fehling's test** [14]: 1ml of Fehling's [A and B in 1:3] was added in 1ml of boiled sample. The presence of carbohydrate was confirmed by the appearance of red precipitate in the given sample solution. **Bradford test** [14]: On boiling water bath 1ml of Bradford's reagent with 1ml of sample was allowed to boil for about 2-3 minutes. The appearance of carbohydrate was confirmed by the appearance of red precipitate in the given sample. **Benedict's test** [14]: On boiling water bath Benedict reagent and 0.5ml of both the sample was heated for minutes. The presence of yellow/orange/green/red, color in the sample. **Foam test** [14]: In a measuring cylinder 50gm of extract is diluted with distilled water and the volume is made up to 20ml by shaking well for 15minutes. The presence of saponin was confirmed by the layer of foam. **Millon's test** [14]: Take 2ml of sample and Millon's reagent. White precipitate gives positive result for proteins. **Biuret test** [14]: In 2ml of sample few drops of 1% $CUSO_4$ Solution, 1ml of 95% ethanol and alcoholic KOH pellets were added. The presence of protein was confirmed by the appearance of pink color in ethanol layer. **Ninhydrin test** [16]: 2drops of Ninhydrin's reagent was added in 2ml of sample. The presence of amino acid was confirmed by the appearance of purple color in the given sample. **Phytosterol test** [14, 15]:500 microlitre of sample was evaporated by heating; the dried extract is used for the test, Libermann-Buuchards method. Dissolved sample in 2ml of acetic acid-anhydride and 2 drops of concentrated was added in the test tube. The change in the color indicates presence of phytosterols. **Ferric chloride** [13, 15]: 5% $Fecl_3$ solution was added to the extract and is

dissolved in 5ml of distilled water. The presence of phenol was confirmed by the appearance of green color. **Lead acetate** [14]: Add 3ml of 10% Lead acetate to 1ml of aqueous solution to dried sample. The appearance of white precipitate is indicative of presence of phenols. **Alkaline reagent** [14, 16]: Add 10% NaOH to aqueous solution to dried sample. The Appearance of yellow fluorescence is a positive test for flavonoids. **Magnesium and HCl** [14, 16]: Add 5ml alcohol to dried extract. Add a few magnesium ribbons followed by drop wise addition of HCl. The color changes from pink to crimson in positive test. **Gum and mucilage** [14]: Take 500 microliter of sample and evaporate the solvent by heating method. Add 10 ml of distilled water and 25ml of absolute alcohols .The presence of gum was confirmed by the appearance of white precipitate.

Antibacterial assay [1, 16]

Mueller Hinton agar plates were prepared. On the underside of the Petri plates four markings are made. The homogenous inoculum of bacteria was made and swabbed on the agar media. Sterile cork-borers (3mm diameter) were used to get wells bored-under aseptic condition. Subsequently 25 µl of extract was pipette into the respective wells. A standard disc was placed as a reference. Control on the previously marked position [17]. Diffusion of the extracts is allowed by keeping the plates at room temperature for 30minutes and then kept for incubation at 37°C for 24 hours.

Antifungal assay [18]

This was done by using well diffusion method. Sabouraud Dextrose agar plates were prepared as per the given composition, autoclaved, poured into sterile petri dishes and allowed to solidify. The plates were labeled with the names of all the fungal strains under aseptic condition. On the underside of the Petri plates four marking were made: three marks for three respective solvent extracts, and one for antifungal disc. A loop full of the selected strain of fungi maintained at 4°C was taken and inoculated into 10 ml of sterile saline solution. Using sterile cotton swab, the suspension was swabbed over sterile pre-cooled plates in order to get lawn growth. The plates were then allowed to dry for 30sec. All fungal plates were maintained at room temperature. The wells were bored into respective marking with the help of sterile cork borer. Extracts were pipetted into the wells (25 µl) and at in room temperature. Antimicrobial test for purified extract was carried out using same procedure.

Results

Qualitative estimation of phytochemicals

The n-hexane extracts of all the test plants has not shown the presence of any of the phytochemicals. The aqueous and methanol extracts of *Datura starmonium* showed the presence of alkaloids, carbohydrates, proteins and phenolic components (Table.1). Aqueous and methanol extracts of *Parathenium* was positive for alkaloids, carbohydrates, sterols and phenolic compounds (Table.1). Whereas methanol extracts of *Lantana camara* was positive for alkaloids, carbohydrates, sterols and phenolic compounds and the aqueous extracts showed positive only to carbohydrates, sterols and phenols (Table.1). Both the aqueous and methanol extracts of *Calotropis-gigantea* was positive only for carbohydrates, sterols and phenolic compounds (Table.1).

Antibacterial assay

Aqueous, methanol and n-hexane extracts of all the four test plants used to check its antibacterial efficacy for the chosen nine bacteria. An antibiotic Penicillin was considered as the standard or control. The highest inhibition zone of 17 mm observed with *Staphylococcus citreus*, whereas the methanol extracts of *Datura starmonium* showed 18.9 mm for the same bacteria. However other test organism has shown the inhibition zone less than 17mmwith Penicillin and the *Datura starmonium* (Table.2). Out of three extracts obtained from four plants the n-hexane extract not shown any inhibition zone for any of the test organism.

Antifungal assay

The highest antifungal activity observed with the penicillin standard with the inhibition zone of 37 mm followed by the aqueous extracts of lantana camera with 12 mm inhibition zone [Table. 3]. The results of antifungal activity are not as promising as the bacterial activity [Table. 2 and 3)

Table 1: Phytochemical analysis of plant extract

TESTS	Plants name											
	Datura			Parthenium			Lantana			Calotropis		
	A	M	H	A	M	H	A	M	H	A	M	H
Mayer's	+	-	-	-	-	-	-	+	-	+	-	-
Wagner's	+	+	-	+	+	-	-	+	-	+	+	-
Herger's	+	-	-	-	-	-	-	+	-	+	+	-
Dragendroff's	+	-	-	-	-	-	-	-	-	+	-	-
Molish	-	+	-	-	+	-	+	+	-	-	-	-
Felhing's	+	-	-	-	-	-	-	-	-	-	-	-
Barfoed's	-	-	-	-	-	-	-	-	-	-	-	-
Benedicts	+	+		+	+	-	+	+	-	-	-	-
Foam	-	-	-	-	-	-	-	-	-	-	-	-
Millon's	+	+	-	-	-	-	-	-	-	-	-	-
Biuret	-	-	-	-	-	-	-	-	-	-	-	-
Ninhydrine	-	-	-	-	-	-	-	-	-	-	-	
Sterol	-	-	-	+	-	-	+	+	-	+	+	-
Ferric chloride	+	+	-	+	+	-	+	+	-	+	+	-
Leadacetate	+	-	-	-	-	-	-	-	-	-	-	-
Alkaline	-	-	-	-	-	-	-	-	-	-	-	-
Mg &HCl	-	-	-	-	-	-	-	-	-	-	-	-
Gum & mucilage	-	-	-	-	-	-	-	-	-	-	-	-
Oils	-	-	+	-	-	+	-	-	+	-	-	+

A-Aqueous, M-Methanol, H-n-Hexane,(+)Presence,(-)Absence

Table 2: Antibacterial activity of extracts against selected bacteria

Plant name	**Solvent**	a	b	C	D	E	f	J	h	I
Standard (Penicillin)		16	17	9	9	9	15	10	11	8
Parthenium	Aqueous	-	-	-	-	-	-	-	7,7	-
	Methanol	6,7	6,6	7,7	-	-	-	-	-	6,7
	Hexane	-	-	-	-	-	-	-	-	-
Daturastarmonium	Aqueous	8,7	-	11,9	-	-	-	7,7	-	9,7
	Methanol	16,16	18,19	15,15	9,10	7,10	-	8,8	-	14,14
	Hexane	7,6	-	11,8	-	-	-	-	-	7,10
Lantana camara	Aqueous	-	-	-	-	-	-	-	-	-
	Methanol	9,9	6,6	9,8	8,8	7,8	-	6,7	10,11	10,11
	Hexane	7,7	-	11,8	-	-	-	-	-	7,6
Calotropis gigantean	Aqueous	-	-	-	-	-	-	-	-	-
	Methanol	6,6	7,7	7,9	7,7	-	-	9,7	-	6,7
	Hexane	-	-	-	-	-	-	-	-	-

a) Staphylococcus aureus, b) Staphylococcus citreus, c) Bacillus aureus, d) Pseudomonas aeruginosa, e) Proteus mirabilis, f) Salmonella typhi, g) Klebsiella, h) Serratia, spp., i) Bacillus polymyxa,

Table 3: Antifungal activity of extracts against selected fungi

Plant name	**Solvent**	a	B	C	d	E	F	G	h	I
Standard (Penicillin)		37	23	10	19	-	-	10	16	-
PartheniumHysterophorus	Aqueous	-	-	7,6	-	8,7	8,9	-	7,7	7,8
	Methanol	6 , 5	-	-	-	7,7	-	-	6,6	-
	Hexane	-	-	-	-	-	-	-	-	-
Daturastarmonium	Aqueous	-	-	-	6,6	-	-	-	-	-
	Methanol	6 ,4	7,5	4,6	-	8,8	-	-	12,14	-
	Hexane	-	-	-	-	-	-	-	-	-
Lantana camara	Aqueous	12,12	7,7	8,9	7,7	7,9	13,9	6,7	-	-
	Methanol	-	-	-	-	-	-	-	8,7	8,7
	Hexane	-	-	-	-	-	-	-	-	-
Calotropis gigantean	Aqueous	-	-	-	-	-	-	-	10,9	-
	Methanol	6,7	6,6	5,6	8,8	7,6	-	-	-	-
	Hexane	-	-	-	-	-	-	-	-	-

a)Candida albicans, b)Candida parapsilosis, c)Cryptococcus, d)Aspergillusoryzae, e)Aspergillusflavus, f)Aspergillusniger, g)Trichophytonmentageophytes, h)Penicillium, Trichoderma, and i)Trichophytonrubrum

Discussion

India's most of the population uses herbs/plant extracts towards illness, even though the presence of modern medicine such as allopathy is predominant but traditional approach towards human health is still having a great importance all over the world [16, 19].Currently the attention of resercher's towards alternative treatment for bacterial and fungal infection has increased due to the trend of antibacterial and antifungal resistance drugs. Plant sources have been provided to be an effective raw material for the extraction of new drugs [15, 16]. The studies also have been reported that these extracts have the active principles available in diluted form which reduces the consequences of overdoses [20]. Previous studies reported that the plants like *Parathenium, Datura starmonium* and *Calotropis-gigantea* have toxic properties and several side effects including inflammation, hepatotoxicity etc., [21, 22]. However small doses were nontoxic when tested on sheep [23], the authors also stated that the aqueous suspension of toxic plants will not

produce any toxicity and can be used safely for therapeutic purposes at specific studied doses [23, 24]. Further report on latex of *Calotropis-gigantea* in 2016 reveals that this plant is having a rich component with the medicinal property for the application in cardiac muscle issues [25]. The present study selected four study plants *Parathenium, Lantana Camera, Datura starmonium* and *Calotropis-gigantea* for the extraction of antimicrobial component. The study observes that all the selected four plant extracts indicated the antibacterial and antifungal activity on test organisms differently depending upon the type of the solvent used for the extraction. In connection to this the earlier studies reported that the type of solvent and its composition plays an important role in separating the phytochemicals responsible for antimicrobial activity [self ref]. The plants always have a potential to produce or synthesize the secondary metabolites such as alkaloids, saponins, flavonoids etc which is responsible for antibacterial activity [14, 15]. This study reveals the presence of alkaloids, carbohydrates, saponins and flavonoids in aqueous and methanol extracts (Table-1). However these all components are absent in n-hexane extract, in comparison with the study report of Hussain et al., showing the n-hexane extracts of *Calotropis-gigantea* were negative for any of the phytochemical content [22].

In the current study, the methanol extract of *Datura starmonium* was very effective against all the five test bacteria but the aqueous extract was useful only against three bacterial species and was inactive against *E.coli* and *A.niger.* The zone of inhibition for the test bacteria ranging from 8 mm to 16 mm for methanol extracts (Table 2). In comparison with reported zone of inhibition 2 to 2.6 cm for *E.coli, S. aureous, B. subtiles* (ref). However, the antifungal study was not much effective (Table 3) in comparision with the bacteria. Whereas the aqueous extract of both *Lantana camara* and *Parthenium hysterophorous* was found effective only against *A.niger*. The methanol extract of *Parthenium hysterophorous* and *Lantana camara* was effective only against *S.aureus* and *B.subtilis* with the zone of inhibition ranging from 7 mm to 9mm (Table-2). Methanol extract of *calotropis gigantea* and *parthenium hysterophorous*was inactive against *E.coli.* The aqueous extract of both the plants were inactive against *S.aureus* also. But the methanol extracts of both plants were effective against *S.aureus.* Other scholers also have indicated the leaf and latex extracts of *Calotropis gigantea* was active with four selected pathogenic bacteria and six important resistant fungi [26], apart from this the methanolic extract of yet an another study was positive to *Salmonella typhi* and *E.Coli* along with gram negative bacteria [24]. According to Gomahetal., 2014 the organic solvent and aqueous extracts of

Calotropis was proved to be effective against Yeast species and few gram negative bacteria [27]. yet an another study conducted in 2018 shows positive for *E.coli* but negative or no inhibition to ***P.aeruginosa***.

Conclusion

The four test plant even though toxic in nature but can be very useful towards medical importance, the study shows the presence of alkaloids, flavonoids and phenolic compounds in aqueous and methanolic extracts whereas the phytochemical compounds are absent in n-hexane extract. The antibacterial effect of *Datura stramonium* was highest compare to the other plant extracts, further study on quantitative estimations on phytochemicals and the standardization of the concentration of extract required for maximum zone of inhibition is required.

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