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COMMON WEED'S PHYTOCHEMICAL ANALYSIS AND *IN-VITRO* ANTIBACTERIAL ACTIVITY

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ABSTRACT: Alkaloids, flavonoids, steroids, and phenolic compounds are among the phytochemicals that have a significant impact on antimicrobial activity. These substances can be derived from several plant components, including the roots, stems, and leaves, and are used to treat bronchitis, cholera, dysentery, fever, and diarrhea. In this work, the phytochemicals from the dried leaves of Parthenium, *Datura stramonium*, and *Calotropis gigantea* are attempted to be extracted. These weeds are frequently found in India and are members of the Asteraceae, Solanaceae, and Apocynaceae families. The presence of phytochemicals, the effectiveness of the antibacterial effect on nine selected bacterial species, and the antifungal activity on eight fungi obtained from the Genohelix lab were all tested using aqueous, methanol, and n-hexane extracts. Aqueous and methanolic extracts from *Datura stramonium* and *Calotropis gigantea* revealed the presence of alkaloids and phenolic compounds, however, n-hexane extracts from all of the test plants failed to detect any phytochemical components. Compared to the conventional antibiotic penicillin, the antibacterial activity of *Datura stramonium* was most efficient against Staphylococcus citrus with a zone inhibition of 18 mm; however, antifungal activity was not as effective.

INTRODUCTION: The very old medical system known as Ayurveda, created more than 5,000 years ago, addresses physical and spiritual wellness. Herbal medicines and cures are valued in Ayurveda [1]. Numerous herbal remedies are employed, including cinnamon and cardamom [2]. Microorganisms cause basic diseases in developing nations, posing a severe public health concern in a notable portion of the population and not being covered by the public or private health care system [3].

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The phytochemicals that are derived from plants are crucial for antibacterial action. These secondary metabolites typically have therapeutic effects on a variety of disorders. Steroids, flavonoids, fatty acids, alkaloids, phenolic compounds, and tannins are examples of metabolites that can affect a patient physiologically. To treat bronchitis, cholera, dysentery, fever, and diarrhea, these substances can be drawn from various plant components, including the roots, stems, and leaves [4].

Parthenium, *Datura starmonium* and *Calotropis-gigantean* are the common available weeds in India and belong to family *Asteraceae*, *Solanaceae* and *Apocynaceae* respectively. *Parthenium* is an abundant source of amino acids, volatile oils, flavonoids, terpenoids, and phenolic compounds. This extract exhibits anti-inflammatory, antipyretic, and analgesic properties [28].

Only a few studies have shown that this weed can also be employed as a substrate for manufacturing bio-surfactants [2]. Datura is a popular marijuana for its pharmacological effects. An excerpt from this document describes how to treat respiratory, dental, skin, and nervous system illnesses. However, this genus is also employed to comprehend hybridity and antimicrobial or antibacterial action [29]. *Lantana camara* is a popular medicine used as anti-plasmodic, carminative and antimetic. This extract shows treatment against cold, bronchitis, asthma and cough. It is also used as hepatotoxic activities, antifungal, antitumor and analgesic [30]. Calotropis eczema, leprosy, rheumatism, syphilis, and lupus. Additionally, the qualities of this plant extract have been proven to have anticoagulant and antibacterial activity [36]. The current investigation aims to evaluate the effectiveness of these plant extracts against pathogenic bacteria and fungus while considering the history of the therapeutic significance of these weeds [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 the outskirts of Bellary and Lantana camara from the 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 blended into a powder and stored for later use. Under sterile conditions, 5gm of each powder sample was dissolved in 50 ml of distilled water. The set-up is centrifuged for 30 minutes at 5000 rpm while being kept at a temperature of 40 and placed in a rotatory shaker for 48 to 72 hours. The supernatant was used for additional tests while the precipitate was discarded.

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°C 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 Dragondroff 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 a boiling water bath, 1ml of reagent with 1ml of sample was allowed to boil for about 2-3 minutes. The appearance of carbohydrates was confirmed by red residue in the given sample.

Benedict's Test [14]: On a boiling water bath, Benedict reagent and 0.5ml of both sample was heated for minutes. The color of yellow/ orange/ green/ red 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 the well for 15minutes. The layer of foam confirmed the presence of saponin.

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. Add 10 ml of distilled water and 25ml of absolute alcohol. The appearance of white precipitate confirmed the presence of gum.

Antibacterial Assay [1, 16]: Plates of Mueller Hinton agar were made. Four marks are created on the Petri plate bottoms. A uniform inoculum of bacteria was prepared and swabbed on the agar media. Wells were bored using sterile cork-borers (3 mm in diameter) in an aseptic environment. After that, 25 l of the extract was pipette into each well. A common disc was set up as a guide. Control in the spot that was previously designated [17]. The plates are left at room temperature for 30 minutes before being incubated at 37°C for 24 hours to allow the extracts to diffuse.

Antifungal Assay [18]: The well diffusion approach was used for this. According to the composition, Sabouraud Dextrose agar plates were made, autoclaved, put onto sterile petri dishes, and allowed to set. All of the fungal strain names were written on the plates in an aseptic environment. Four markings were created on the underside of the Petri plates: one mark for the antifungal disc and three marks for the three different solvent extracts. The chosen strain of fungi was extracted from a loop that had been kept at 4°C and injected into 10 ml of sterile saline solution. The suspension was swabbed with a sterile cotton swab over sterile, previously cooled plates to induce lawn growth. The plates were then given a 30-second drying period. The temperature of each fungus plate was kept at room temperature. Using a sterile cork borer, the wells were drilled into their corresponding markings. 25 l of the extract was pipetted into the wells at room temperature. The purified extract underwent an antimicrobial test using the same methodology.

RESULTS:

Qualitative Estimation of Phytochemicals: None of the phytochemicals have been detected in the n-hexane extracts of any of the test plants. Alkaloids, carbohydrates, proteins, and phenolic components were detected in the *Datura starmonium* aqueous

and methanol extracts **Table 1**. Alkaloids, carbohydrates, sterols, and phenolic substances were detected in *Parathenium* extracts in both aqueous and methanol **Table 1**. While *Lantana camara* methanol extracts were positive for alkaloids, carbohydrates, sterols, and phenolic compounds, aqueous extracts only tested positive for those three substances **Table 2**. Only carbohydrates, sterols, and phenolic substances were detected in *Calotropis gigantea's* aqueous and methanol extracts **Table 1.**

Antibacterial Assay: The antibacterial effectiveness of the four test plants was examined using aqueous, methanol, and n-hexane extracts of the selected nine bacteria. The standard or control was the antibiotic *Penicillin*. *Staphylococcus citreus* demonstrated the largest inhibitory zone of 17 mm, whereas *Datura starmonium* methanol extracts showed 18.9 mm for the same bacteria. However, the inhibition zone with Penicillin and Datura starmonium was less than 17mm with other test organisms **Table 2**. The n-hexane extract, one of three derived from four different plants, did not exhibit any inhibitory zones for any test organisms.

Antifungal Assay: The highest antifungal activity was observed with the penicillin standard with an inhibition zone of 37 mm, followed by the aqueous extracts of the lantana camera with 12 mm inhibition zone **Table 3**. Antifungal activity results are not as promising as the bacterial activity in **Tables 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	-	-	-	-	-	-	-	-	-
Datura starmonium	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	-
Parthenium hysterophorus	Aqueous	-	-	7,6	-	8,7	8,9	-	7,7	7,8
	Methanol	6 , 5	-	-	-	7,7	-	-	6,6	-
	Hexane	-	-	-	-	-	-	-	-	-
Datura starmonium	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) *Aspergillus oryzae*, e) *Aspergillus flavus*, f) *Aspergillus niger*, g) *Trichophyton mentageophytes*, h) *Penicillium, Trichoderma, and* i) *Trichophytonrubrum.*

DISCUSSION: Although allopathy is the most common kind of modern medicine, most people in India still utilize herbs and plant extracts to treat illnesses [16, 19]. Due to the rise in antibacterial and antifungal drug resistance, researchers are now paying more attention to alternate treatments for bacterial and fungal infections. It has been demonstrated that plant sources can serve as a useful raw material for extracting novel medicines [15, 16]. According to studies, these extracts have the active ingredients available in diluted form, which lessens the effects of overdosing [20].

According to earlier research, plants like *Parathenium*, *Datura starmonium*, and *Calotropis-gigantea* have poisonous qualities and several adverse effects, such as hepatotoxicity and inflammation [21, 22]. The authors also suggested that the aqueous suspension of hazardous plants will not generate any toxicity and can be used safely for therapeutic reasons at particular studied levels [23, 24], even though modest doses were not toxic when tested on sheep [23]. According to a 2016 paper on *Calotropis-gigantea* latex, this plant has a rich component with therapeutic properties that can be used to treat cardiac muscle problems [25]. To extract an antibacterial component, the current study used four study plants: *Parathenium*, *Lantana camera*, *Datura starmonium*, and *Calotropis gigantea*. According to the study, the type of extraction solvent used impacted how each of the four plant extracts showed antibacterial and antifungal activity on test organisms. According to past research, the type of solvent and its makeup are crucial factors in isolating the phytochemicals responsible for antibacterial action [self ref]. Alkaloids, saponins, flavonoids, and other secondary metabolites important for antibacterial activity can always be produced or synthesized by plants [14, 15].

In the aqueous and methanol extracts, this investigation found the presence of alkaloids, carbohydrates, saponins, and flavonoids **Table 1**. However, Hussain *et al.* report demonstrating that the n-hexane extracts of *Calotropis gigantea* were devoid of any phytochemical content [22] shows that all of these components are absent from the n-hexane extract. In the current study, the methanol extract of *Datura starmonium* was very effective against all five test bacteria. Still, 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 ranges from 8 mm to 16 mm for methanol extracts **Table 2**. Compared with a 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 comparison with the bacteria. Whereas the aqueous extract of both *Lantana*

camara and *Parthenium hysterophorous* was found effective only against *A. niger*. Only *S. aureus* and *B. subtilis* were resistant to the methanol extract of *Parthenium hysterophorous* and *Lantana camara*, with a zone of inhibition extending from 7 mm to 9 mm **Table 2**. *Calotropis gigantea* and *Parthenium hysterophorous* methanol extract did not affect *E. coli*. Both plants' aqueous extracts were ineffective against *S. aureus*. However, both plants' methanol extracts were efficient against *S. aureus*. In addition, the methanolic extract of yet another study was positive for *Salmonella typhi* and *E. coli*, along with gram-negative bacteria [24]. Other researchers have also suggested that the leaf and latex extracts of *Calotropis gigantea* were active with four important resistant fungi and four pathogenic bacteria [26]. The effectiveness of the organic solvent and aqueous extracts of Calotropis against several gram-negative bacteria and yeast species was demonstrated by Gomahetal in 2014 [27]. Another 2018 study demonstrates positive results for *E. coli* but negative or no suppression of P. *aeruginosa.*

CONCLUSION: The study demonstrates the presence of alkaloids, flavonoids, and phenolic compounds in the aqueous and methanolic extracts, while the phytochemical compounds are lacking in the n-hexane extract of the four test plants, even though they are poisonous by nature. *Datura stramonium* had the strongest antibacterial impact compared to other plant extracts; further research is needed to standardize the concentration of extract needed to achieve the maximal zone of inhibition and quantitative estimations of phytochemicals.

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CONFLICT OF INTEREST: The article entitled Common weed's phytochemical analysis and *in-vitro* antibacterial activity is here with submitted for publication in International Journal of Pharmacognosy (Name of Journal). It has not been published before, and it is not under consideration for publication in any other journal (s). I/We certify that I/We have obtained written permission for the use of text, tables, and/or illustrations from any copyrighted source(s), and I/We declare no conflict of interest.

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