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Phytochemical screening and antibacterial activity of leaves of *Eclipta alba* (L.) Hassk on important species of Bacteria

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Abstract

In vitro evaluation of antifungal activity of aqueous extract of *Eclipta alba* (Leaves) were tested against four gram negative bacteria at 10µl-100 µl concentration. In *E. coli* maximum and significant activity was observed in 80-100 µl concentration tested and recorded 2.0mm to 34.0mm inhibition. In *K. pneumonia*, the percent inhibition was up to 25.0 mm inhibition. In *P. aeruginosa* the inhibition percentage was in the range of 1.0mm to 21.0mm respectively. In *S. typhi* maximum inhibition was observed and recorded 35.0mm inhibition in 100 µl concentration and significant activity was also observed in 80 and 90 µl concentration. All the result was compared to standard antibiotics Chloramphenicol (25mg) and Gentamicin (25mg) and recorded 28.0mm to 35.0mm inhibition in all the test bacteria.

Keywords: *Eclipta alba*, phytochemical analysis, Gram negative bacteria, antibacterial activity

Contents

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1. Introduction

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Antony et al., 2008) A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials (Khalaf et al., 2007). In the ancient India, medicinal plants were used to prevent various critical diseases. The plant kingdom is an important source of herbal drugs. Even in recent years, there has been an increasing awareness about the importance of medicinal plants. Generally, herbal drugs are easily available, safe,

less expensive, efficient, and rarely have side effects. According to World Health Organization, medicinal plants would be the best source to obtain variety of drugs (Shreya et al., 2013). Use of medicinal plants as a drug is alternative method for the management of pathogenic microbes like bacteria, fungi and viruses and is co-friendly. Number of studies had been conducted worldwide to prove the antimicrobial efficacy of traditional used medicinal plants (Thenmozhi and Rajeshwari, 2010; Sharma and Kumar, 2009). In the recent decades, the interest in evaluating therapeutic effects of plants has increased dramatically [Thuille et al., 2003; Alanís AD et al., 2005] as 80% of the world's people rely on complementary and alternative medicine for their health care needs [Magee, 2005; Duraipandiyan et al., 2006]. Phytoplants have been shown to be good alternatives to synthetic chemical antimicrobial agents and antibiotics because of the serious side effects, antimicrobial resistance and the emergence of previously uncommon infections that have been reported to be on the increase due to inappropriate or widespread overuse of antimicrobials [Pawar and Nabar , 2010; Olila et al., 2001].

2. Materials and Methods

Plant Materials:

Healthy leaves of *Eclipta alba* (L.) Hassk were collected from western Gahts, Karnataka. The plant materials were washed thoroughly 2-3 times with running tap water and once with sterile distilled water, air dried at room temperature on a sterile blotter and used for preparation of extract. (Satish et al., 1999)

Phytochemical Analysis:

Tannins: 200 mg of leaves of *E. alba* was macerated in 10ml of distilled water. 2 ml of filtrate was treated with 2 ml of FeCl3 (Ayoolla et al., 2008).

Alkaloids:

200 mg of leaves of *E. alba* was macerated in 10ml of methanol and filtered. 2 ml of filtrate was treated with 1% HCl and steam, filtered. To 1ml of obtained filtrate add 6 drops of Mayor‟s or Wagner‟s reagent and dragendroff reagent (Ayoolla et al., 2008).

Saponins: Exactly 0.5 ml filtrate of leaves of *E. alba* was added to 5 ml of distilled water and warm (Frothing test) (Ayoolla et al., 2008).

Cardiac glycosides: 2 ml of filtrate of leaves of *E. alba* filtrate was added to 1 ml of glacial acetic acid, 1 ml of FeCl3 and 1 ml of concentrated H2SO4 (Liebermann-Burchard reaction) (Ayoolla et al., 2008).

Flavonoids: 200 mg of leaves of *E. alba* was macerated in 10 ml of ethanol, filtered. 2 ml of filtrate was treated with 2 ml of conc. HCl and 2 ml of magnesium (Ayoolla et al., 2008).

Reducing sugar: 0.5 ml of *E. alba* extract was added to 1ml of water and 5-8 drops of hot Fehling‟s solution. Brick red precipitate was observed. (Ayoolla et al., 2008).

Terpenoids: The leaf extract of *E. alba* was mixed with 2ml of chloroform and concentrate H_2SO_4 (3ml) is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive result of the presence of terprnoids.(Dharmendra et al., 2012)

Phenol: Few drops of neutral 5% ferric chloride solution. A dark green colour indicates the presence of phenolic content. (Saxena, 2012).

Extraction

Aqueous extraction:

50 grams of thoroughly washed leaves of *E. alba* were macerated with 50 ml of sterile distilled water in a waring blender (waring International, New Hartford, CT, USA) for 10 min. The macerate was first filtered through double-layered muslin cloth and then centrifuged at 4000 gram for 30 minutes. The supernatant was filtered through Whatman No.1 filter paper and sterilized at 120 ^{0}C for 30 minutes. The extract was preserved aseptically in a brown bottle at 5^0 C until further use (Gupta et al., 1996; Pinto et al., 1998).

Antibacterial activity assay:

Test pathogens:

In vitro antibacterial activity was examined for aqueous extracts. Authenticated pure cultures of four Gram negative human bacteria viz., *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Klebsiella pneumoniae.*were obtained from Research Center, CMR Institute of Management Studies (Autonomous), Bangalore. All bacteria were grown in Mac Conkey agar medium and maintained at 4^0C until further use.

Preparation of Inoculum

Preparation of standard culture inoculums of test organism:

All the test bacterial species were inoculated into 2 ml Mac Conkey broth and incubated at 37^0C for 24 hours till the growth in the broth was equivalent with Mac-Farland standard (0.5%) as recommended by WHO [Magee, 2005].

Antibacterial assay

Aqueous Extract

Agar cup diffusion method: An overnight culture of *E. coli, P. aeruginosa, S. typhi* and *K. pneumonia* was standardized to contain approximately 107cfu/ml. Bacteria was inoculated into 20 ml of Mac Conkey broth and allowed to set. Thereafter, all the inoculum was swabbed over the surface of the Mac Conkey agar medium plate using sterile cotton swab. Using a sterile cork borer of 5 mm diameter, five wells were made in solidified sterile Mac

Conkey agar medium (one in the centre and four wells at the corner). The agar plugs were removed with a flamed and cooled wire loop. Then 10,20,30,40, 50, 60, 70, 80, 90 and 100µl of aqueous extract of *E. alba* roots were placed in the wells made in inoculated plates. The treatment also includes 50 µl of sterilized distilled water as control. All the plates were incubated for 24hours at 37^0C and zone of inhibition if any around the well were measured in millimeter (mm). For each treatment ten replicates were maintained. The same procedure were followed for standard antibiotics Chloramphenicol (25mg) and Xanthomycin (25mg) to compare the efficacy of aqueous extract against test organisms [Alanís et al., 2005; Duraipandiyan, 2006].

Table 1: Phytochemical screening of *Eclipta alba* (L.) Hassk

Phytochemicals	Test	Reactions	Present/Absent
Tannins	Ferric chloride test	Blue-black precipitate indicated the presence of Tannins	+
Alkaloids	Mayer,s test Dragendroff‟s test Wagner‟s reagent	Creamish precipitate brownish-red precipitate orange precipitate indicated the presence of respective alkaloids	+
Saponins	Frothing test	Frothing persistence on warming indicated presence of saponins	+
Cardiac glycosides	Keller-Kiliani test	Green-blue colour indicated the presence of cardiac glycosides	-
Steroids	Liebermann-Burchard reaction	Blue-green ring indicated the presence of terpenoids	+
Flavonoids	NaOH test	Ribbon pink-tomato red color indicated the presence of flavonoids	+
Reducing sugar	Fehling‟s test	Brick red precipitate	+
Terpenoids	Salkowski Test	A reddish brown coloration	+
Phenols	Ferric chloride test	Dark green colour	+

3. Result and Discussion

Result

Phytochemical Analysis:

Among the nine phytochemical constituents tested, *E.alba* showed the presence of tannins, alkaloids, steroids, flavonoids, reducing sugar, saponins, terpenoids and Phenols. Cardiac glycosides were not identified in the leaves of *E.alba* when subjected to standard conformation test (Table1).

Antibacterial assay:

Antibacterial activity of leaves of *E.alba* showed maximum inhibition of *E. coli* at 100µl concentration and recorded 34.0mm inhibition. 32.0mm in 90 µl, 29.0 mm in 80 µl, 27.0mm in 70 µl concentration. In*K. pneumonia* at 100 µl concentration, the percent inhibition was 25.0mm, at 90 µl it was recorded 22.0mm, 20.0mm in 80 µl concentrations. In *P. aeruginosa* maximum inhibition was recorded at 100µl concentration and showed 21.0mm inhibition and at 80 µl concentration, it was recorded 18.0mm inhibition. In *S. typhi* maximum inhibition was in 100 µl concentration and recorded 35.0mm inhibition. 32.0mm inhibition was observed in 90 µl concentration, 29.0mm in 80 µl concentration. Compared to syntheitic antibiotics Chloramphenicol and Gentamicin tested at 25mg, all the test pathogens recorded 28.0mm to 35.0mm inhibition (Table 2).

Discussion

Literature survey reveals that many plants have been evaluated *in vitro* for their antifungal potency against some important species of bacteria. Similarly, some plants have been evaluated for their antibacterial potency i*n vitro* against some plant pathogenic bacteria. The results of the present investigations clearly reveals that the aqueous extract of the leaves of *E.alba* are antibacterially active. In the present investigation, the leaves have been tested for antibacterial activity against some important pathogenic bacteria. When it is tested at 10 to 100µl concentration it is showing a promising result against all the bacteria.

Compared to synthetic antibiotics Chloramphenicol (25mg) and Gentamicin (25mg) the leaves of *E.alba* showed a similar result where the synthetic antibiotics can be avoided which is responsible for different types of side effects. This has driven plant pathologists to search for alternative eco-friendly methods for the management of plant diseases. Traditionally, plants have been exploited by man for the treatment of human diseases (Sharma et al., 1999; Kitula, 2007). However, not much information is available on the exploitation of plants for the management of plant diseases. Plant based remedies would be of immense value in developing ecofriendly remedies due to their easy biodegradability, less phytotoxic and more systemic nature [Sharma et al., 1999]. Hence using medicinal plants to cure both plant and human diseases is an alternative method to avoid the side effects of synthetic drugs.

Table 1: Antibacterial activity of aqueous extract of leaves of *Eclipta alba* (L.) Hassk

Bacteria	Zone of inhibition(mm)											
	Concentration											
	Plant extract										Synthetic antibiotics	
	10 µl	20 µl	30 µl	40 µl	50 µl	60 µl	70 µl	80 µl	90 µl	100 µl	Chloramphenicol (25mg)	Gentamicin (25mg)
E. coli	2.0^{a} ±0.0	5.0^{b} ±0.0	9.0^{c} ±0.1	16.0^{d} ±0.0	20.0^{e} ±0.1	23.0^{f} ±0.1	27.0^{g} ±0.0	29.0^{h} ±0.0	32.0^{i} ±0.0	34.0^{j} ±0.1	35.0^{b} ±0.2	32.0^{a} ±0.2
K. pneumonia	0.0^{a} ±0.0	1.0^{a} ±0.1	4.0^{b} ±0.0	9.0^{c} ±0.1	12.0^{d} ±0.0	15.0^{e} ±0.1	17.0^{f} ±0.0	20.0^{g} ±0.0	22.0^{h} ±0.1	25.0^{i} ±0.0	30.0^{a} ±0.3	33.0^{b} ±0.2
P. aeruginosa	1.0^{a} ±0.0	3.0^{b} ±0.1	6.0^{c} ±0.0	8.0^{d} ±0.0	10.0^{e} ±0.1	13.0^{f} ±0.1	16.0^{g} ±0.0	18.0^{h} ±0.1	21.0^{i} ±0.1	21.0^{j} ±0.1	32.0^{b} ±0.2	30.0^{a} ±0.1
S. typhi	2.0^{a} ±0.1	5.0^{b} ±0.1	7.0^{c} ±0.1	13.0^{d} ±0.2	16.0^{e} ±0.1	21.0^{f} ±0.1	25.0^{g} ±0.0	29.0^{h} ±0.0	32.0^{i} ±0.0	35.0^{j} ±0.0	30.0^{b} ±0.1	28.0^{a} ±0.1

- Values are the mean of ten replicates, ±standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.
- Pattern of percentage inhibition increase is not uniform for all the microorganisms

4. Conclusion

Form this observation, it can be concluded that, the leaves of *E.alba* recorded a promising result against all the test fungi and all the phytochemical present and identified in phytochemical analysis has to be purified and future work is necessary to test each constituent for antibacterial activity and to isolate bioactive compound.

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