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Phytochemical Screening of Methanolic Extract and Antibacterial Activity of *Eclipta alba and Morinda citrifolia* L.

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Abstract: Aerial parts of combined crude extracts *Eclipta alba and Morinda citrifolia L.* are used traditionally for the treatment of several diseases of liver, skin and stomach. Extract and active principle compound of a well known Indian hepatoprotective herb, *Eclipta alba and Morinda citrifolia L.* was tested for *In vitro* antimicrobial studies. It was evaluated using zone of inhibition studies and minimum inhibitory concentration. The extract exhibited activity against all eight strains studied. Preliminary phytochemical analysis of extracts revealed the presence of antimicrobial compounds such as alkaloids, flavonoids, tannins, saponins etc. Among the test samples ethyl acetate extract showed pronounced antimicrobial activity, while ethanol extract exhibited the least activity and petroleum ether extract failed to inhibit the test pathogens. Based on the observations, *Eclipta alba and Morinda citrifolia L.* appears to be a valuable source for antimicrobial principles.

Key words: Antibacterial · Antifungal · *Eclipta alba* · *Morinda citrifolia* L.

INTRODUCTION

Phytochemical analysis of plants, used in folklore has yielded a number of compounds with various pharmacological activities. In view of the increasing development of resistant microorganisms, treatment of various diseases caused by microorganisms has become a major challenge in the human medical field. This may be due to the synthetic nature of these substances, but also to their known side effects and in some cases to their unpleasant smell, taste or the burning sensation felt on the skin. Medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents. About 3,000 materials from 2,764 plant species have been screened for their pharmacological and chemotherapeutic properties [1]. Various biological activities are possessed by *E. alba,* such as memory disorders treatment, general tonic, edema, fevers and rheumatic joint pains treatment, digestion, hepatitis, enlarged spleen, antioxidant activity and skin disorders [2-4]. *Morinda Citrifolia* is one of the most important traditional Polynesian medicinal plants. This small evergreen tree is native from South Eastern Asia to Australia and now it has a Pantropical distribution. It has antifungal, antibacterial, anti-inflammatory and antiviral activities. *Morinda citrifolia L.* was studied for its antimicrobial activity. *Morinda citrifolia* is an important medicinal plant which has been used for many centuries through out the south pacific. It is a small shrub, three to twelve meters height. The leaves, seeds, bark, green fruit and roots have been used in various tropical remedies in diverse parts of the South East Asia and Pacific Islands. Most pacific islands medicinal plants have yet to be studied due to geographical isolation from the western world. Their potential therapeutic properties are still unknown. The leaves are used to treat cough, nausea and colic, possibly due to its anti inflammatory activity. The leaves have also been used to treat gout, tuberculosis and ring worm. In the Philippines, the seeds are eaten in order to expel intestinal worms. This plant has also been popular as a source of red, yellow and purple dyes [5-7]. Morinda is reputed to have antibacterial, antiviral, antifungal, antitumor, antihelminthic, analgesic, hypotensive, anti-inflammatory and immune enhancing

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effects. Originally, the leaves were applied directly to the skin to treat ulcerations and minor infections. Some marketing companies have proposed that drinking noni juice can be used against a very wide variety of ailments [8-11].

The main aim of the present investigation was to study the antimicrobial activity and preliminary phytochemical screening of combined *Eclipta alba* and *Morinda citrifolia L.* leaf, extract in different solvent like absolute alcohol, chloroform, acetone.

MATERIALS AND METHODS

Collection of Plant Material: Mature leaves of of *Eclipta alba and Morinda citrifolia L.* were collected from Ater Dist. Bhind, Madhya Pradesh India during April 2010. Collected material was washed thoroughly in running tap water, rinsed in distilled water and shade dried in open air and grounded into powder.

Extraction Procedure: The leaves were dried under shade and coarsely powdered. The powder was successively extracted using soxhlet apparatus with ethanol and water. These extracts were condensed using rotary vacuum evaporator followed by vacuum evaporator and stored in desiccator. The powder of all the extracts was suspended in appropriate solvent systems and was subjected to further analysis.

Phytochemical Screening: The aqueous, ethanol and acetone extracts of *Eclipta alba and Morinda citrifolia L.* were screened for the presence of secondary metabolites using the procedure. One milliliters of each extract was measured into a test tube for each of the tests and concentrated by evaporating the extractant in a water bath. Tests were carried out for carbohydrates, reducing sugars, tannins, polyphenols, lipids, flavonoids, ketones, alkaloids, steroids.

Determination of Alkaloids: A measured weight of the sample was dispensed into 10% acetic acid solution in ethanol to form a ratio of 1:10. The mixture was allowed to stand for 4 hours at 28°C. It was later filtered with filter paper and the filtrate was treated with drop wise addition of aqueous NH_4OH until the alkaloid was precipitated, this was washed with 10% ammonia solution and dried in the oven at 80°C.

Determination of Flavonoids: A 5g of the sample was boiled in 50ml of 2M HCl solution for 30 minutes under reflux. It was allowed to cool, then filtered through filter paper and the filtrate was treated with equal volume of ethyl acetate.

Determination of Tannin: A 5g portion of the sample was dispensed in 50ml of distilled water and mixed properly. This was allowed to stand for 30 minutes at 28°C before it was filtered. 2 ml of the plant extract was dispensed into a 50ml volumetric flask. Similarly, 2ml standard solution and 2ml of distilled water were put in separate volumetric flask. The reagent was added to each of the flask and 2.5 ml of saturated Na_2CO_3 solution was also added, the total content of the flask was made up to 50ml with distilled water and incubated at 28°C for 90 minutes. A spectrophotometer set at 260 nm wavelength was used to measure the respective absorbance using the reagent blank to calibrate the instrument.

Determination of Steroid: A measured weight of the sample was dispensed in 100ml freshly distilled water and homogenized in laboratory blender. This was filtered and was eluted with normal ammonium hydroxide solution (pH 8). 2ml of the eluate was put into the test tube and mixed with 2ml of chloroform. 3ml of ice-cold acetic anhydride were added to the mixture in the flask and 2 drops of concentrated H_2SO_4 were added to cool. Standard sterol solution was prepared and spectrophotometer at 310 nm was used to measure the absorbance.

Test Concentrations: The crude extracts were dissolved in Dimethyl sulphoxide (DMSO) and extracts were loaded on the 6 mm dia. sterile disc (Himedia, Bombay) with the concentrations of 1.25, 2.5 and 5 mg/disc.

Antimicrobial Assay: The bacterial cultures were maintained in Nutrient Agar (NA) and fungal cultures were maintained in Sabouraud Dextrose Agar (SDA) slants at 4°C. The bacterial cultures were inoculated in Mueller Hinton (MH) broth and incubated at 37°C for 10 h at 600 rpm. The bacterial inoculum was standardized to 1.5 OD at 387 nm and it was used for disc diffusion method. The final inoculum size of $2x10^8$ CFU/ml for bacteria and $2x10^4$ CFU/ml for *Candida* were used for

broth micro dilution technique. Antifungal screening was carried out by broth micro dilution method; the final inoculum size was 2×10^4 spores/ml.

Determination of Antimicrobial Properties of the Crude Extract by Agar Diffusion Method: Preliminary antibacterial screening was carried out using disc diffusion method [12]. Discs with different concentrations of plant extracts were placed on the preinoculated Mueller Hinton Agar (MHA) plates with respective cultures and were incubated at 37°C for 24 h. 1ml of the test organisms (24 hours old culture) was aseptically injected into sterile plates. 20ml sterilized nutrient agar (NA) was poured on top of the test organism aseptically after it has been allowed to cool to about 45°C. The medium was mixed gently for even distribution of the inoculums within the media and allowed to solidify at room temperature (25°C). Sterile cork borer of 8mm in diameter was used to make five (5) wells on the solidified agar into which 0.5ml extracts of the sample (water and solvent extracts) were aseptically introduced into the well separately with the aid of syringe and it was labeled. A control experiment was set up with well containing standard antibiotic; ciprofloxacin (10 µg/disc) and DMSO were used as positive and negative control, respectively. The inhibition zone around the disc (diameter) was measured and recorded. The plates were incubated at 37°C for 24 hrs. Zones of inhibition were observed around each well after 24 hrs and recorded appropriately against the extract. Results were quoted as the radii (mm) of the zone of the inhibition around the well. In the fungi isolates, 5 ml of the extract was impregnated with 25 ml of sterile potato dextrose agar (PDA) and allow to setting at 25°C. A sterile 8 mm cork borer was used to inoculate the fungi isolate at the centre of the plate. The plates were incubated at 27°C for 72 hrs. The radical growth of the fungal isolates was recorder at every 24 hrs.

Minimum Inhibitory Concentrations (MIC): Broth micro dilution method was used to determine the MIC. This was carried out in 96 well microtitre plates containing 200 µl Mueller Hinton Broth with different concentrations of plant extracts. The final concentration of DMSO was maintained at 0.1% in the test broth. Triplicates were maintained along with the negative control. Plates were incubated at 37°C for 12 h for bacteria and at 27°C for fungi. MIC was determined as the complete inhibition of growth at lowest concentration.

$$\% \text{ growth inhibition} = \frac{\text{Mycelia growth in the control} - \text{Mycelia growth in the treated sample}}{\text{Mycelia growth in the control}} \times 100$$

RESULT AND DISCUSSION

The phytochemical analysis of *Eclipta alba and Morinda citrifolia L.* showed the presence of different groups of secondary metabolites viz., alkaloids, tennin, flavonoids, terpenoids, which are of medicinal importance. Of the test extracts, ethanol extract showed positive results for most of the test compounds. The phenolic group and flavonoids were rich in ethyl acetate extract when compared to other metabolites (Table 1). The results on antimicrobial potency of the *Eclipta alba* and *Morinda citrifolia L.* extracts against eight microbial strains are presented in Table 2 and 3. The antimicrobial activity was assessed using the agar disc diffusion method by measuring the diameter of growth inhibition zones with concentration of different solvent extracts. The results showed that the ethyl acetate extract exhibited broad spectrum of inhibition zones against *Klebsiella pneumoniae* (21 mm), *Micrococcus luteus* and *Candida albicans* (16 mm), *Salmonella typhimurium* (11 mm), *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (each 19mm). The ethanol extract showed feeble activity against the test organisms (between 6-8 mm). However, ethyl acetate extract strongly inhibited a Gram positive bacterium (*Micrococcus luteus*) and Gram negative bacteria (*Klebsiella pneumoniae* and *Salmonella typhimurium*) at 244 µg/ml (MIC value) concentration. The results obtained from the disc diffusion assay showed that there has been an increasing effect on microbial growth inhibition with increasing concentration of the extract. The extract showed good inhibitory activity on almost all the microbes tested.

Table 1: Preliminary phytochemical screening of *Eclipta alba and Morinda citrifolia L.* extracts

	Extracts			
Compound	P	EA	E	W
Alkaloids	-	+	+	-
Anthracene glycosides	+	-	+++	+
Tannin	-	+	+	-
Flavonoids	-	+++	+	-
Terpenoids	+++	-	-	+

Extracts: P: Petroleum ether; EA: Ethyl acetate; E: Ethanol; W: Water
Phytochemical tests: +++: quantitative; +: positive; -: negative; T: trace

Table 2: Antimicrobial properties of *Eclipta alba and Morinda citrifolia L.* extracts

Organisms	Extract Concentration µg/disc	Extracts EA	Extracts E	Standards*
Bacillus cereus	250	14	11	Ciprofloxacin
Micrococcus luteus	250	12	15	Ciprofloxacin
Staphylococcus aureus	250	17	12	Ciprofloxacin
Escherichia coli	250	14	14	Ciprofloxacin
Pseudomonas aeruginosa	250	19	11	Ciprofloxacin
Klebsiella pneumoniae	250	21	11	Ciprofloxacin
Salmonella typhimurium	250	24	14	Ciprofloxacin
Candida albicans	250	16	17	Ciprofloxacin

Extracts: EA: Ethyl acetate; E: Ethanol

Table 3: Minimum inhibition concentrations of *Eclipta alba* and *Morinda citrifolia L. extracts*

Organisms	Extracts µg/ml EA	Extracts µg/ml E
Bacillus cereus	344	177
Micrococcus luteus	216	298
Staphylococcus aureus	258	476
Escherichia coli	437	553
Pseudomonas aeruginosa	376	455
Klebsiella pneumoniae	200	542
Salmonella typhimurium	200	548
Candida albicans	334	754

Extracts: EA: Ethyl acetate; E: Ethanol

The maximum inhibition was recorded against *E. coli* with the extract of petroleum ether in 20mm.The Gram positive *S. aureus* was susceptible with the inhibition zone ranging from 18mm in water extract. The maximum inhibition was observed against *C. albicans* with the chloroform extract. The water extract showed significant effect against *Klebsiella pneumoniae*. The observed activity may be due to the presence of potent phytoconstituents in the extract. This may be indicative of a significant potential for isolating purer compounds. Antimicrobial activity of *Eclipta alba* and *Morinda citrifolia L.* extract is compared with the antibiotics` of the respective organism. It was found that the extract in some cases exhibited the zone of inhibition which was equal or greater than the zone of inhibition of antibiotic [13]. As a result it is` sure that these leaf extract can surely inhibit the growth of these microorganisms there by preventing various disease such as skin infections, diabetes, cancer etc. *Eclipta alba* and *Morinda citrifolia L* extract thus provides safe, easy, effective and practical solutions to every day ailments leaving behind no toxins and creating a clean, pleasant atmosphere.

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