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Research Article

EVALUATION OF PHYTOCHEMICAL CONSTITUENTS AND ANTIBACTERIAL PROPERTIES OF *AGERATUM CONYZOIDES* LINN., AGAINST THE MOST COMMON SKIN INFECTION CAUSING AGENTS

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ABSTRACT

Phytochemical constituenys in plants have a variety of uses and may even serve as anti-microbials. Our study is aimed at evaluating the phytochemical constituents of A.conyzoides by qualitative analysis and also to evaluate the antibacterial properties of the extracts of A.conyzoides by disc diffusion method. The extracts of the plants contain all the phytochemicals that were tested for and the extracts of the plant possessed significant antibacterial properties at concentrations of 50mg/ml and more. The ethyl acetate extract of the plant has the highest zone of inhibition activity among all the forms of extracts of A.conyzoides.

Keywords: Ageratum conyzoides, antibacterial property, ethyl acetate extract, Phytochemicals, Disc diffusion method.

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INTRODUCTION:

In the developed countries and more affluent regions, phyto-pharmaceutical therapy is being used as an alternative to biomedicine and also for the treatment of mild and chronic health problems. Here the sustenance of conventional medicine is being ensured by combining the complementary medicine and biomedicine, as in the case of cancer therapy where in there are many side effects of chemo therapy in cancer and in such cases, complementary medicine is being used to treat the collateral symptoms of chemotherapy. Also there is a rise in the consumption of plant based nutraceuticals as a way of change in fashionable life-styles. Apart from this there is also another part of the developed world that doesn't encourage experimenting with the plant based medicines anymore in case of severe of acute ailments. There are regions among the third world countries even today that its population don't have access to modern medicine and still rely upon the age old traditional medicine like the Ayurveda, Unani, and Siddha systems of treatment for the treatment its ailments. [1] Since the beginning of the human civilization, plants have been the primary sources of drugs and the use of medicinal plants for healing predates the recorded history and every day new evidences are emerging that still push this recorded history even further in time. In fact even the modern medicine is dependent on plants for its medicine, a few

instances- digoxin is used in cardiac problems and is obtained from digitalis leaf, quinine is an antimalarial drug and is obtained from cinchona bark, morphine is being used in palliative care in terminally ill cancer patients and this is obtained from opium poppy, aspirin is being used as an analgesic and also as antiplatelet drug and this is obtained from opium poppy. [2] Globally 80% of the population is dependent on medicines obtained from plants for its primary health care issues and also for the alleviation of symptoms arising from ill health as these products are free from undesirable effects. There is also an ever growing demand for these plant based products in health care, nutrition, and in cosmetic industry. [3] These days the primary focus of global pharmaceutical industry is on plant based research for medicine and also regulatory bodies around the globe like- in the USA "NATIONAL CENTER FOR COMPLIMENTARY AND ALTERNATIVE MEDICINE" , are spending billions of dollars to identify the potential medicine from plants. [4,5,6]

As per 2014 data, about 4% of patients who had undergone inpatient surgery in the United States. In the United States, Surgical Site Infections are the leading contributors of health care associated infections. According to an estimate, each year over 157,000 cases of surgical site infections occursuffered from post-operative surgical site infections. In a study published in the Journal of Patient Safety Study, it was concluded that each year 440,000 patients die from medical errors in hospitals that can be prevented. Another study published in the Journal of Healthcare Finance, it was estimated that $ 19.5 billion is the cost of burden imposed due to medical errors in the year 2008. [7]

Microbes are usually cloistered at the skin surface and gain entrance into the underlying tissues once the skin is wounded. The class of the wound whether having contaminated, or colonized or locally infected or critically colonized or invasive infection depends on the state of infection and replication of the microbe. The presence of non-replicating organisms in the wound tissue is called as contamination, and on the other hand the presence of replicating microbes in the wound without damaging the tissue is called as colonization. Critical colonization or local infection is an intermediate state with the starting of local tissue responses due to microbe replication. Invasive infection may be defined as the presence of replicating microbes in the wound with ensuing host injury. In the process of infecting a wound, the bacteria conglomerate and form a bio-film by secreting an extracellular polysaccharide matrix. The bio-films that are mature develop protected microenvironments and are more resistant to conventional antibiotics. The commonest species of bacteria found in both infected and non-infected wounds are- *S. aureus*, *P. aeruginosa*, and *β-Hemolytic streptococci*.[8]

Aims & Objectives:

The aim of our study is to evaluate A.conyzoides for phytochemical constituents and also to evaluate the antibacterial properties of the plant against the most common skin infection causing organisms.

Objectives:

i. To identify the Phytochemical constituents of the extracts of *Ageratum cnyzoides* L., by Qualitative Analysis.
ii. To assess the antmicrobial property of extracts of *A.conyzoides* against common skin infection causing pathogens.
iii. To estimate the zone of inhibition (ZOI) of the extracts of *A.conyzoides* against common skin infection causing pathogens.

MATERIALS & METHODS:

The chemicals necessary for conducting the screening of phytochemicals were obtained from SD fine chemicals, Chennai. The bacterial cultures were obtained from MTCC, Chandigarh. Discs and media were obtained from Himedia, Mumbai.

Preparation of plant material: the plants were picked from around the University, got confirmed at the Department of Botany, Sunrise University, Alwar, Rajasthan. Then the whole plant is dried in shade for 14 days and pounded to coarse power with mallet process. This coarse powder was used to prepare extracts of water, hydroethanolic, methanolic, and ethyl acetate. These extracts were used for evaluating phytochemical constituents and antibacterial properties.

Qualitative analysis of phytochemical constituents: The crude extracts were subjected to phytochemical qualitative tests and the compounds tested for were- alkaloids, terpenoids, saponins, tannins, and flavonoids. The general methods available for qualitative analysis of these compounds are as follows:

Terpenoids: a small amount of each of the extracts were mixed with 5 ml of chloroform and to each of the test tubes, 3 ml of 0.1 M H_2SO_4 was added. A reddish brown colour at the junction of 2 liquids indicates the presence of terpenoids.

Alkaloids : a small amount of each of the extracts were dissolved in 5 ml of ammonia and the solutions were fractionated with 5 ml of chloroform. The chloroform fraction was removed and to it 3 ml of dilute hydrochloric acid was added and to the acid layer a few drops of Drgendroffs reagen was added. Development of reddish brown precipitate indicates the presence of alkaloids.

Tannins : a small amount of each of the extracts were boiled with 10 ml of distilled water and filtered. To each of the filtrates, a few drops of 1% ferric chloride was added. Appearance of blue-black colouration indicates the presence of tannins.

Flavonoids: a small amount of each of the extracts were mixed with 10 ml of methanol and to each of the mixtures, a few drops of neutral ferric chloride solution was added. Appearance of green color indicates the presence of flavonoids.

Saponins : around 500 mg of each of the extracts were boiled with 10 ml of distilled water and shanken vigorously to produce a stable persistent froth which indicates the presence of saponins.

Determination of antibacterial activity (Disc diffusion method) : The organisms that are commonly involved in skin infections are E.coli, S.aureus, P.aeruginosa. These bacteria are tested for susceptibility against all the extracts of A.C.

Preparation of plant extracts for antibacterial testing: 100 mg of each of the plant extracts were dissolved in 1 ml of sterile distilled water and labeled as per their solvet names. Now each ml of these stock solutions contain 100 mg/ml. Further, this stock solution of 100 mg/ml was used to prepare solutions of concentrations 50%, 25%, 12.5% of all the extracts by serial dilution methods. Now these freshly prepared solutions contain 100 mg/ml (stock solution), 50 mg/ml, 25 mg/ml, and 12.5 mg/ml of the extracts.

Preparation of bacterial culture: The following bacterial cultures were used for the study- Staphylocccus aureus (MTCC 3160), Escherichia coli (MTCC 443), Pseudomonas aeruginosa (MTCC 741). The S.aureus obtained was phenol coefficient test organism and is in nutrient agar growth medium.

The revival of the bacteria was done by mixing them with 0.5 ml of broth and then incubated at 34^0C. MHA media was prepared and poured into the plates of 100mm diameter and allowed to cool. Now 100 µl of the active bacterial culture was transferred to the MHA plates and allowed to dry for 10 minutes. The plates were labeled as per the solvents in the extracts (hydro, hydroethanolic, methanolic, and ethyl acetate). The testing of antibacterial properties was done in triplicates.

Preparation of discs for antibacterial activity testing: The sterile discs of diameter 6mm were loaded with 100 µl of each of the extract solutions of concentrations 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml and were allowed to dry. Then these discs were placed on the MHA plates that were inoculated with the bacteria. Now these plates were incubated at 34^0C for 24 hours. After incubation period, the zoi around each disc in the MHA plates were recorded.

RESULTS & DISCUSSION:

Qualitative analysis for phytochemical constituents of A.C:

The entire plant was dried and pounded into coarse powder. 250 g of this coarse powder was used for extraction process by using hydro, hydroethanolic , methanolic and ethyl acetate solvents. The extracts were concentrated and stored. For the purpose of phytochemical screening, the extracts were used separately and screened for phytochemicals, in particular, alkaloids, flavonoids, saponins, tannins, and terpenoids. The results obtained were as follows:

Table- 1 phytochemical constituents in different extracts of A.C

s.no.	Constituent	Hydro extract	Hydro-ethanolic extract(50/50)	Methanolic extract	Ethyl acetate extract
1	Alkaloids	√	√	√	√
2	Terpenoids	√	√	√	√
3	Flavonoids	√	√	√	√
4	Tannins	√	√	√	√
5	Saponins	√	√	√	√

The extracts of entire plant of Ageratum conyzoides exhibited the presence of alkaloids, terpenoids, flavonoids, tannins, and saponins. The presence of these compounds makes the plant a potential lead plant for evaluating of wound healing properties. It is a known fact that tannins are **polyphenols** and are infact astringent and it also a known fact that **phenols** are free radical scavengers. Hence phenol containing compounds are considered to be antioxidants. It is also logical to say that antioxidants are anticancer compounds.

Flavonoids are polyphenolic secondary metabolites that possess a wide array of pharmacological activities, for instance they can be used as protectants, as anticancer drugs and likewise. The presence of flavonoids in A.C confirms its use as wound healing plant in traditional medicine.

Alkaloids are substances with a number of uses, prominent among alkaloids are Hyoscine, ergot alkaloids etc. the presence of alkaloids in A.C was confirmed and this in turn validates the traditional use of A.C in folk medicine.

Terpenoids are used in many different ways some examples are: as insect propellents, as antifeedants, as phytoalexins, as attractants of pollingranes, as pheromones, as defensive substances against herbivorous animals, as allelochemicals, as signal molecules, and also as plant growth hormones.

Saponins are natural glycosides with a variety of uses like – detergents, expectorants, vasoprotective, anti-inflammatory, antiparasitic, immunomodulatory, hypocholesterolemic, molluscicidal, hypoblycaemic, antifungal, they can be used as sweeteners and emulsifiers, as adjuvants in production of vaccines, as raw materials in the production of steroidal hormones.

Evaluation of antimicrobial properties of Ageratum conyzoides:

The discs loaded with different concentrations of the plant extracts were placed on the MHA plates and incubated for 24 hours at 34^0C. Then these plates were removed and the zoi around each disc was measured using a ruler. The observations were as follows:

Table 2 the mean of zoi (in mm) of different extracts at different strengths against various bacteria.

	Hydro extract in mg/ml				Hydroethanolic extract in mg/ml				Methanolic extract in mg/ml				Ethyl acetate extract in mg/ml			
	12.5	25	50	100	12.5	25	50	100	12.5	25	50	100	12.5	25	50	100
S. aureus	8	10	13	15	5	8	10	12	9	12	12	14	12	16	19	24
E.coli	5	6	8	11	6	7	9	12	8	11	13	16	9	15	20	25
P.aeroginosa	na	na	5	11	na	6	10	13	5	7	9	15	7	10	14	18

*** *na = no activit*

The values in the table 2 are obtained by rounding off the mean zoi values in mm to the nearest value. As can be witnessed from the table, the highest concentration of all the extracts (100 mg/ml) exhibited the highest zoi values in all the bacterial species under study. The ethyl acetate extract exhibited the highest zoi towards all bacterial species under study at all the study concentrations. It was also observed that all the extracts of A.C are capable of inhibiting the microbial species under at concentrations of more than 25 mg/ml. of the three bacterial species under study, both S.aureus and E.coli exhibited the highest sensitivity towards ethyl acetate extract of A.C.

CONCLUSION:

Our study was aimed at evaluating the phytochemical constituents, wound healing properties of Ageratum conyzoides Linn., and also to improve the antimicrobial for skin infections. The results obtained in our study have confirmed the presence of all important phytochemical constituents in A.C, and hence validates the claims of traditional medicine of various medicinal uses of A.C. The presence of flavonoids gives strength to the claim that A.C can be used as a protectant and as wound healing agent, the presence of tannins gives strength to the claim that A.C can be used as an antioxidant, the presence of glycosides validates the claim as an insect repellant, and further the presence of saponins even strengthens the claim further that A.C is an anti-inflammatory and and anti-fungal agent.

The wound healing properties of A.C. was reported by many authors and was also confirmed by our study and moreover the antibacterial activity was also confirmed by our study. The ethyl acetate extract of A.C in particular is active against the most common bacteria that causes skin infection, viz., S.aureus, E.coli, P.aeroginosa. The ethyl acetate extract of A.C appears promising in effectively controlling the invading bacteria in post surgical wounds and in controlling skin infections. A.C possesses both the wound healing and antibacterial properties, so this inherent property makes

it ideal for developing into a compound that can be used post surgically and also pre-surgically for effectively reducing the hospital stay and also for fighting infections post surgically.

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