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Phytochemical screening and antimicrobial activities of leaf extracts of *Ageratina altissimia (*Asteraceae)

Nagendrababu Kunisetti

Gokul College of Pharmacy, Piridi, Bobbili, Vizianagaram dist, Andhra Pradesh

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ABSTRACT

The experimental work is carried out for evaluate the photochemical Analysis and antimicrobial activities of Aqueous, Chloroform, Carbontetrachloride, Ethylacetate, Ethanolleaf extracts of Ageratina altissimia plant. Photochemical screening of the plant extracts showed the presence of various compounds like Alkaloids, Flavanoids, Carbohydrates, fats & fixed oils, steroids. The antimicrobial screening was carried out by two Gram +ve (Staphylococcus aureus NCIM 2079, Bacillus subtilis NCIM 2063) and three Gram –ve (Escherichia. coliNCIM2065 Pseudomonas aeruginosa NCIM 2036KlebsiellaaerogenesNCIM 2098) bacteria and fungi (Candida albicans NCIM 3102, Aspergillus niger NCIM 1054)[1] using agar diffusion methods {disk diffusion method for Aqueous extract; well diffusion method for solvent extracts} for bacteria and poisoned food technique for fungi compared with standard drug. MICs of the crude extracts were determined by broth macrodilution method. All the microorganisms were markedly affected by these extracts. The chloroform extract exhibited the lowest MIC value against Staphylococcus aureus (125 µg/ml) and (150 µg/ml) against Aspergillus niger .Finally, conclude that the plant having natural antiseptic action

Keywords: Phytoconstituents, Leaf extracts, Antibacterial and Antifungal activity, *Ageratina altissimia*, agar disk diffusion method, agar well diffusion method, poisoned food technique.

INTRODUCTION

Medicinal plants are the foundation of many important drugs of the modern world. Plants are now playing an important role in many medicines like allopathic medicine, herbal medicine, homoeopathy and aromatherapy. In nature many plants and plants seed provided source of medicine at the earlier times. Plants have proven to be the most useful in curing diseases and provide an important source of pharmacy and medicine. Plants have great significance to the health of individuals [2]. Herbal drug constitutes a major part in all the traditional system of medicines [3].The *Ageratina altissimia* belongs to Family of ASTERACEAE Plant Kingdom & well distributed in world wide. An older binominal name for this species was *Eupatorium rugosum.* Eupatorium genus has many more species. It is also called as white snake root and having many medical uses like diarrhea, kidneystones, fever and antidote for snake bite poising. The medicinal importance of these plants lies in some chemical substances that produce a distinct physiological action on the body of human. The major importance of these bioactive constitute of plants are Steroid, Terpenoids, Tannins, Carotenoids, Flavonoids, Alkaloids and Glycosides. Antibacterial activity is method to destroying or suppressing the growth or reproduction of bacteria. The term antibacterial terms derives from Greek word ―anti‖ that means against. The compound which destroys or suppresses the growth or reproduction of bacteria, and that type of compound or agent having such properties is called antibacterial agent or

antibacterial compounds. These are the either drugs or any plants material that destroy or inhibit the growth of Bacteria. Chemotherapeutic agents also having ability to prevent or treat bacterial infections. The main purpose of my study is to screen secondary metabolites that are photochemical analysis and to estimate the antibacterial activity of *Ageratina altissimia*.

Antibacterial activity is done *in vitro* by agar disk diffusion method by using distilled water, ethanol as solvents and agar wall diffusion method by using chloroform, ethanol, ethyl acetate, calcium tetrachloride as a solvents and these extract are use against bacteria (e.g.: *Escherichia coli, Klebsiella Pneumonia, staphylococcus, baccilus species etc) and* poisoned food technique for *fungi (aspergilus niger, candida albicans)*. The selection of crude plant extract for screening the antibacterial activity has the potential of being more successful in the initial steps than screening of pure compounds [4].Measure zone of inhibition using Agar diffusion and poisoned food technique methods. Minimum inhibitory concentration is the lowest concentration of an anti-microbial that inhibits or kills the visible growth of microorganisms. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. MIC of the crude extracts was determined by broth macro dilution method.

MATERIALS AND METHODS

Sample collection
The fresh leaves of *Ageratina altissimia* were collected from Bobbili, vizianagram district of Andhrapradesh.

Culture collection
Bacterial and fungal cultures collected from Department of Microbiology, Andhra University.

Preparation of Extracts
The powdered sample was extracted in soxhlet apparatus successively with Water and Ethanol respectively due to their nature of polarity and maceration process with Chloroform, Carbon tetrachloride, Ethyl acetate.

Photochemical Evaluation
The major secondary metabolites such as terpenoids, steroids, flavonoids, alkaloids, saponins and glycosides and primary metabolites like carbohydrates, proteins, fats and fixed oils were screened according to the common photochemical methods.[5][7][10]

Alkaloids
Tannic acid test**:** Leaf extract was treated with tannic acid, a buff color precipitate is formed.

Wagner's test: Leaf extract was treated with Iodine-potassiumiodated solution, a reddish brownish ppt formed.

Flavanoids
Zink HCl test: Leaf extract treated with zinc dust and con HCl, red color is formed.

Fates and Fixed oils
Saponification test: leaf extract treated with 0.5N KOH, Phenolphthalein indicator heat for 1-2 hours, give soap type solution or partial neutralization of alkali.

Carbohydrates
*Molish test***:** leaf extract treated with alcoholic alpha naphthol and con H_2SO_4, purple to violet color ring appeared at the junction.

Selivanoff's test: leaf extract treated with crystal of Resorcinol and con HCl and heat, rose color formed.

Test for pentose's: leaf extract treated with HCl and phloroglucinol and heat, red color is formed.

Test for steroids and tri terpenoids
Salkowski test: leaf extract treated with con H2SO4, red color at lower end indicates steroids and yellow color indicate tri terpenoid.

ANTIMICROBIAL EVALUATION

A. Agar disc diffusion method [8] [9] [12] [14]

Dry and sterilized filter paper discs (6 mm diameter) were impregnated with known amounts of the test substances using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs (Kanamycin 50µg/disc) for Bacteria) and blank discs (impregnated with solvents) were used as a positive and negative control. These plates were then kept at low temperature (4ºC) for 24 h to allow maximum diffusion. The plates were then incubated at 37ºC for 24 h to allow maximum growth of the organisms.

B. Agar well method [6]

Inoculums suspension was spread over the agar plates using sterile L-shaped glass rod. Well of 0.5 cm in diameter was made in inoculated media plate and 150 µl extracts of *Ageratina altissimia* were aseptically filled into the well. The plates were placed at room temperature for an hour to allow diffusion of extract into the agar. Then the plates were incubated for 24 hrs.at 37˚c.

C. **Poisoned food technique [9]**

Potato dextrose agar contain fungus culture was cut with a sterile cork borer and transferred aseptically upside down at the center of Petridish. Suitable checks were maintained, where the culture discs were grown under same conditions on PDA without extract. Solvent checks were maintained to check out the inhibitory effect of solvent on fungi in which PDA was mixed with solvent (a solvent, which is used for dissolving extracts) using standard AMPOTERICIN B (100 µg/disc). Petri plates were incubated at 25 ±1 Growth of fungus colony was measured after every 24h till the fungus in the control plates completely occupied it.

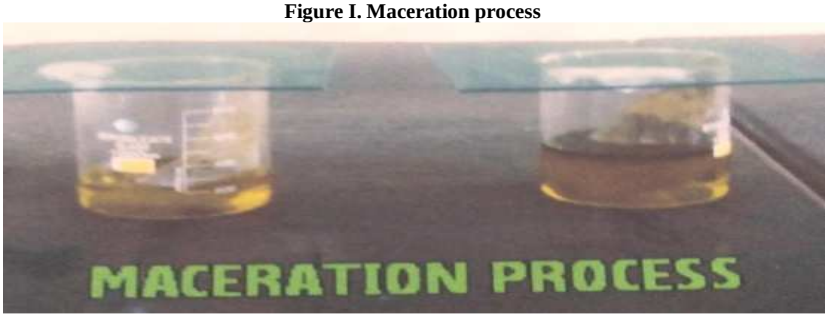
D. Broth dilution method

Quantitative analysis of Antibacterial effect of Petroleum Ether, chloroform, ethyl acetate, c*arbontetrachloride, water* extract of *Ageratinaaltissimia* were determined by Broth dilution method. 1ml of plant extracts was added to 10 ml nutrient broth in 20 ml test tubes. The tubes were than inoculated with appropriate test bacteria and incubated at 30˚c ±1.

RESULTS AND DISCUSSION

In the present study, the photochemical screening and antibacterial activities [8] were performed with Aqueous, Chloroform, Carbon tetrachloride, Ethyl acetate, Ethanol extracts of the leaf of *Ageratina altissimia*. The study was made against two gram positive pathogenic bacteria and three gram negative bacteria using the diffusion methods. The leaves of *Ageratina altissimia* were rich in carbohydrates, flavonoids, alkaloids, steroids and tri terpenoids.

Figure I. Maceration process



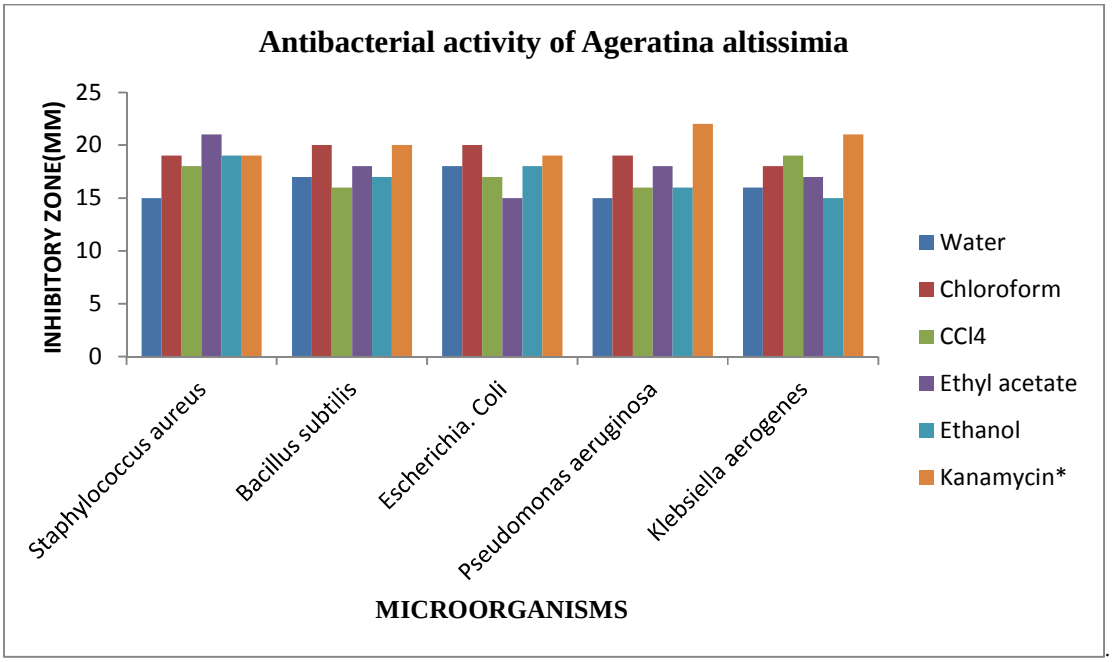
The various photochemical compounds detected{Table 1} are known to have beneficial importance in Medicinal sciences. For instance, flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities [13]. The flavanoids have demonstrated anti-bacterial activity [6].

Dry powdered leaf extracts of *Ageratina altissimia*[7] applied on nutrient media, obtained zone of inhibition results were compared with the standard antibiotics like Kanamycin (50µg/disc) and Amphotericin B (100µg/disc) in agar

diffusion methods and poisoned food technique. Chloroform extract shows the maximum inhibition against microorganisms.

Figure II. Extraction of chloroform, CCl4, Ethyl acetate

Figure. III. Antibacterial activity of crude leaf extracts from *Ageratina altissimia*



All the crude extracts (1000 µg/disc) exhibited moderate to good antibacterial activity against the bacterial pathogens tested here in and the largest zone of inhibition (21 mm in dm) was recorded against Staphylococcus *aureus bacteria and for Aspergillus Niger fungi(55 mm in dm)* {Table 2}. Antibacterial antibiotic Kanamycin* (20 µg/disc) was also found to be active against all the bacteria tested here in.

Minimum inhibitory concentration is the lowest concentration of an anti-microbial that inhibits or kills the visible growth of microorganisms [12]. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. MIC is measured by broth macro dilution method.

The chloroform extract exhibited the lowest MIC value against S*taphylococcus aureus* (125 µg/ml) and *Aspergillus niger(150* µg/ml*)*{Table 3}.It appears that crude leaf extract of has antibacterial and antifungal properties and can be used as a novel antimicrobial agent [9].

Figure. IV. Antifungal activity of crude leaf extracts from *Ageratina altissimia*

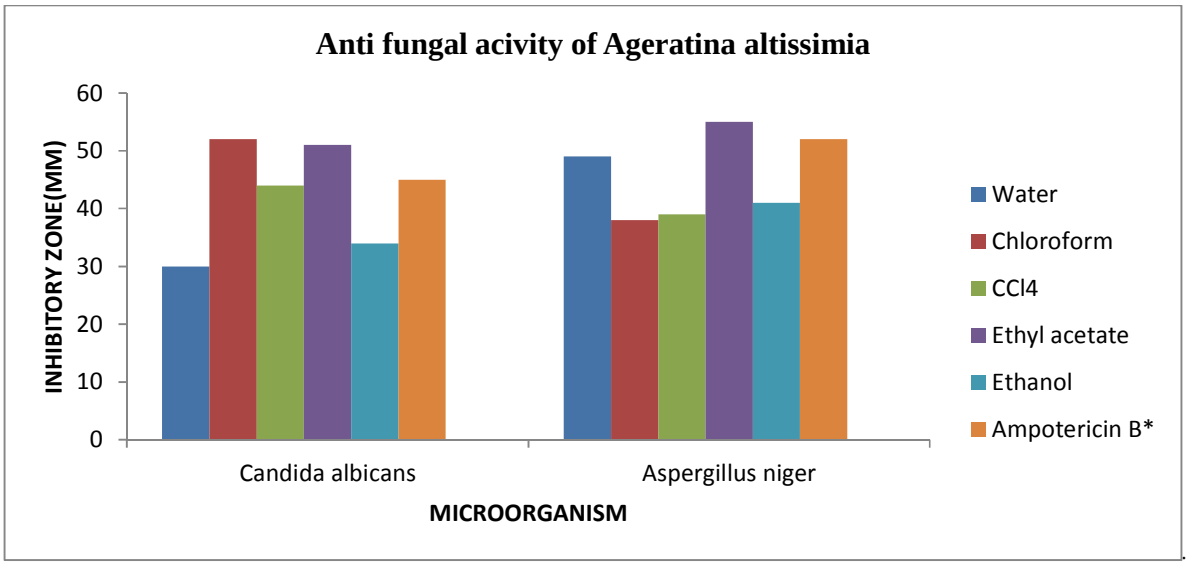


Figure.V. MICs of crude leaf extracts from *Ageratina altissimia* against Bacteria and fungi

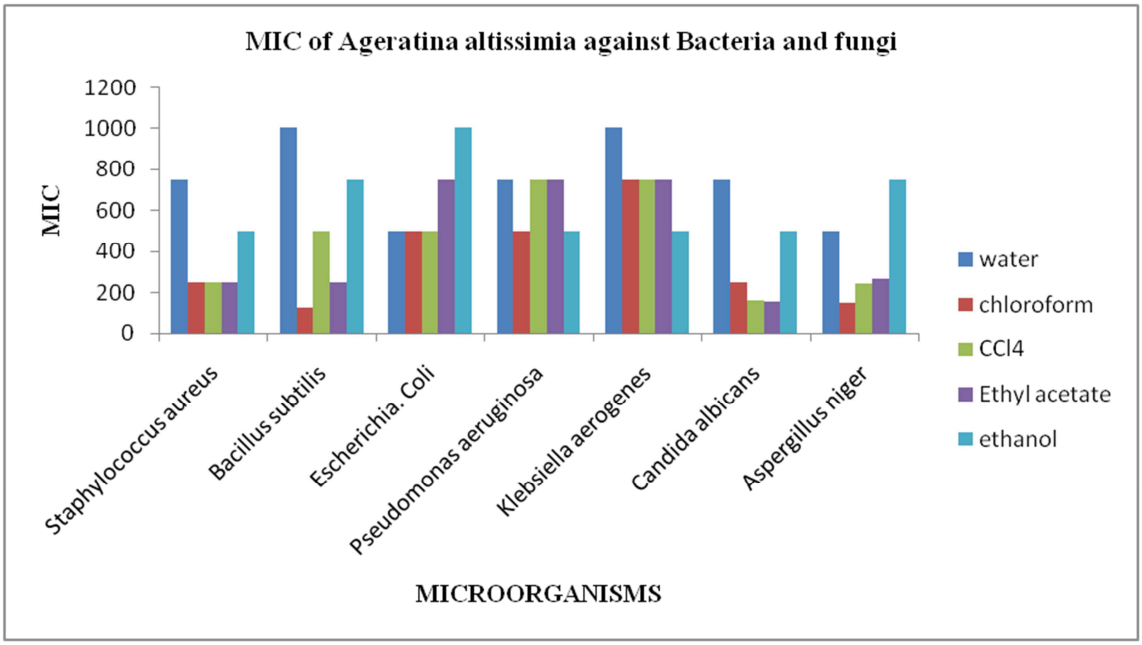


Table I: Preliminary photochemical screening of various leaf extract of *Ageratina altissimia*

Phytoconstituents	Water	Chloroform	CCl_4	Ethyl acetate	Ethanol
Alkaloids	-	+	+	+	+
Amino acids	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Carbohydrates	+	+	+	+	+
Catechins	-	-	-	-	-
Coumarins	-	-	-	-	-
Flavonoids	-	+	+	+	-
Gums, oils and resins	-	-	-	-	-
Proteins	-	-	-	-	-
Phenolic groups	-	-	-	-	-
Quinones	-	-	-		-
Saponins	-	-	-	-	-
Steroids	-	+	+	-	+
Tannins	-	-	-	-	-
Terpenoids	-	+	+	+	-

+ = present; - = absent

Table II. Antibacterial and fungi activity of crude leaf extracts from *Ageratina altissimia*

Bacteria	Diameter of zone of inhibition (mm) (Crude extract 1000 µg/disc)					
	Water	Chloroform	CCl_4	Ethyl acetate	Ethanol	Kanamycin*
Staphylococcus aureus	15	19	18	21	19	19
Bacillus subtilis	17	20	16	18	17	20
Escherichia. Coli	18	20	17	15	18	19
Pseudomonas aeruginosa	15	19	16	18	16	22
Klebsiellaaerogenes	16	18	19	17	15	21
Fungi	**Percentage inhibition of fungal mycelia growth (radius in cm) (100 µg/ml medium)**					
	Water	**Chloroform**	**CCl_4**	**Ethyl acetate**	**Ethanol**	**Ampotericin B***
Candida albicans	30	52	44	51	34	45
Aspergillus niger	49	38	39	55	41	52

Table III. MICs of crude leaf extracts from *Ageratina altissimia* against Bacteria and fungi

Bacteria	MIC (crude extract µg/ml medium)				
	Water	Chloroform	CCl_4	Ethyl acetate	Ethanol
Staphylococcus aureus	750	125	250	250	500
Bacillus subtilis	1000	250	500	250	750
Escherichia. Coli	500	500	500	750	1000
Pseudomonas aeruginosa	750	500	750	750	500
Klebsiellaaerogenes	1000	750	750	750	500
Fungi	**Water**	**Chloroform**	**CCl_4**	**Ethyl acetate**	**Ethanol**
Candida albicans	750	250	165	155	500
Aspergillus niger	500	150	245	265	750

CONCLUSION

Aqueous, Chloroform, Carbon tetrachloride, Ethyl acetate, Ethanol extracts of the leaf of *Ageratina altissimia* were rich in carbohydrates, flavonoids, alkaloids, steroids and tri terpenoids.

Dry powdered leaf extracts of *Ageratina altissimia* uptained zone of inhibition results were compared with the standard antibiotics, Chloroform extract shows the maximum inhibition against microorganisms. Means lowest MIC value (125 µg/ml) against *Bacillus subtilis and against Aspergillus niger(150* µg/ml*)* {Table 2}.Finally, conclude that crude leaf extract of has antibacterial and antifungal properties and can be used as a novel antimicrobial agent

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