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***Ageratum conyzoides* L.: In vitro antimicrobial, antioxidant and phytochemical study**

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ABSTRACT

Ageratum conyzoides L. (Asteraceae) is an aromatic weed. The weed has been known since time immemorial for its therapeutic properties and has been utilized for treatment of various ailments, such as burns and wounds, for antimicrobial properties, for many infectious conditions and bacterial infections, arthrosis, headaches and dyspnea, pneumonia, analgesic, anti-inflammatory, antiasthmatic, antispasmodic and haemostatic effects, stomach ailments, gynaecological diseases, leprosy and other skin diseases. A wide range of chemical compounds including alkaloids, coumarins, flavonoids, chromenes, benzofurans, sterols and terpenoids have been isolated from this species. Present study aimed at qualitative and quantitative phytochemical analysis of leaves of *A. conyzoides*. Phytochemical analysis revealed the presence of tannin, alkaloids, flavonoids, phenols, glycosides, cardiac glycosides, reducing sugar and saponin and absence of anthraquinone, carotenoids and free anthraquinone in all the extracts varying quantities. The plant extracts exhibited the presence of a high amount of phenolics and flavonoid content which might be the key candidates for the antioxidant potential of the extract. The antibacterial activity of *A. conyzoides* was tested against seven bacteria.

Key words : *Ageratum conyzoides*, Antimicrobial activity, Antioxidant Activity, Phytochemical screening, Total Flavonoids, Total Phenol

Introduction

India along with its rich cultural heritage is also known for its abundance knowledge in traditional herbal medicine. The traditional knowledge is based on necessities, requirements, observation, study, instincts or experiments and experience (Jain, 2004). Since the earliest days of civilization, mankind have been directly or indirectly depended on plant resources (Sousa *et al.*, 2021). Even now most of the pharmaceutical industries depends on plants for the synthesis of many important drugs occupying a significant spot. Many of these plants are wild in nature. Although synthetic drugs and antibiotics have brought about a revolution in controlling different

diseases but it is still out of reach for those living in remote areas. People of those areas depends on traditional healers and Ojha whom they trust more. Judicious use of medicinal plants can cure deadly diseases that have long defied synthetic drugs.

Plants are a source of many chemical ingredients. People have been using plants without having any knowledge about the active compounds. If the active ingredients of plants are known, then the mode of action of plants producing therapeutic effects can also be better investigated. The crude drug used contains some beneficial, some harmful and some toxic components, but all integrated under certain natural rule to make the crude fraction into a single chemical agent. These crude drugs have compo-

nents amalgamated in a fashion where one chemical counter balance the undesirable side effect of the other, ultimately aimed to provide beneficial effects (Choudhury *et al.*, 2012).

Various studies have revealed that the plants contain some chemicals not related to their growth and development and were regarded as by-products. But now these chemicals are known as secondary plant metabolites which are required for self-defense in case of plants. Secondary metabolites are also used in anti-feeding activity, toxicity, or acting as precursors to physical defense mechanisms. The most important compounds include alkaloids, terpenoids, steroids, phenols, glycosides and tannins which are distributed in the different plant parts such as roots, leaves, tubers, rhizomes, fruit, stem etc. Human use these secondary metabolites as medicines, flavouring and recreational drugs (Egwaikhide *et al.*, 2009).

The present study aims to determine the secondary metabolites present in different solvent extracts of the plant and their antioxidant and antimicrobial activity. It is already known to us that the phytochemical activities of the plant have been documented from different parts of the world and their activities varies from place to place and are depend on climatic and edaphic factors. The present study was carried out on the sample collected from Dibrugarh, from where no previous study was documented.

Ageratum conyzoides belongs to the family Asteraceae. Asteraceae is well marked family in their characteristics which clearly distinguishes it from the other families. The name *Ageratum* is derived from the Greek word 'Ageras', meaning non-aging, referring to the longevity of the whole plant while *conyzoides*, on the other hand is derived from the Greek word 'konyz', the Greek name of *Inulahelenium*, which the plant resembles (Khare, 2008; Chopra *et al.*, 1956). In Assam, it is locally known as 'Gendhali bon'.

Ageratum consists of approximately 30 species but only a few of them have been phytochemically investigated (Burkill, 1985).

Ageratum is a tropical and sub-tropical plant that is very common in Asia, Africa and South America. The plant is an erect, annual, branched, slender, hairy and aromatic herb which grows to approximately 1m in height. The leaves and stem is covered with fine white hairs. The leaves are stalked, ovate, 4-10cm long and 1-5cm wide with tip and base

slightly pointed with long round toothed margins. The flowers are purple to white, less than 6mm across and arranged in close terminal inflorescences. The fruit is black and are easily dispersed while the seeds are photoblastic and often lost within 12 months. The plant generally grows on waste and ruined sites. It has a strong and peculiar odour that resembles a male goat found in Australia and hence its name 'goat weed' or billy goat weed (Thorat *et al.*, 2018).

Ethnomedicinal uses of *Ageratum*

The essential oil obtained from plant has been reported to have a powerful nauseating odour and found to be poisonous to rabbits due the presence of HCN and coumarin. The herb is not consumed as a vegetable but it is eaten for medicinal purpose. In some culture it is widely used as fodder for guinea pigs, horses, cattle and also used to feed fish. Due to the presence of pyrrolidine alkaloids, they are known to be hepatotoxic. They are of biological importance due their association with Lepidoptera. In India it is used for the treatment of leprosy and as an oil lotion for purulent ophthalmia. The decoction and infusion of the herb is effective in stomach ailments such as diarrhea, dysentery, intestinal colic, flatulence, rheumatism, fever and to relieve pain associated with navel in children. The leaves are used for application on cuts, sores as anti-inflammatory agent, haemostatic, insecticide, in headache, in boils skin diseases, ringworm infection, in typhoid, as an antidote to snake poison in malarial fever, as anti-tetanus, for urine problems, prolapse of anus, for swollen piles, in throat infection, painful gums, on abscess for early suppuration, in wound healing and leucorrhoea. The root is used as an antilithic and in infant diarrhea. It has been reported to have nematocidal activity and have potential use in controlling pests. It is the only plant used in HIV/AIDS disease (Sharma and Sharma, 1995). Plant juice is applied twice daily to cure red eye or conjunctivitis (Srivastava, 2009)

Materials and Methods

Collection and identification of plant sample

Fresh plant sample of *Ageratum conyzoides* was collected from the Dibrugarh University campus of Dibrugarh district. The fresh leaves were sundried and then grinded into fine powdered in

mixergrinder. The powdered sample was carefully kept in clean paperbags and preserved at 4 °C to carry out further analysis.

Preparation of plant extracts

Using four different solvents (Methanol, Ethyl acetate, hexane and chloroform) organic extracts of the plant was prepared. Soxhlet apparatus was used for extract preparation from carefully weighed plant sample at respective temperature. The extract was filtered and concentrated in rotary evaporator. The semi-solid mass was lyophilized and then stored in air tight bottles.

Qualitative phytochemical screening

The different qualitative chemical tests were performed for establishing phytochemical profile of methanol, ethyl acetate, hexane and chloroform. The test for alkaloids, phenol, flavonoid, saponins, phlobatannin, tannin, glycosides, cardiac glycosides, anthraquinone, free anthraquinone, reducing sugar and carotenoids.

Estimation of total phenols

The extracts were dissolved in 5 ml of distilled water and estimated by Folin- Ciocalteau reagent method with absorbance measured at 650 nm with catechol (50 µg/ml) as the standard (Sadasivam 1996)

Estimation of total flavonoids:

The extract was dissolved in DMSO and estimation was done using aluminium chloride method with absorbance measured by 510 nm with quercetin (100 µg/ml) as the standard.

Determination of Antioxidant activity

DPPH radical scavenging activity (Stanojevic *et al.*, 2009). The DPPH method is based on the spectrophotometric measurement of DPPH concentration changes that results from the DPPH reaction with an antioxidant. Absorbance was noted at 517 nm using UV-Vis spectrophotometer. Ascorbic acid was used as standard sample.

ABTS radical scavenging activity: ABTS was carried out using modified method as per Re *et al.* (1999). The absorbance was taken at 734 nm using the spectrophotometer.

Determination of antimicrobial activity

Agar well diffusion method was employed for de-

termination of antimicrobial activity as described by Nair *et al.* (2005) using 6mm borer. The diameter of the zone of inhibition exhibited by the extract determined the antimicrobial activity. All the plates were examined for any zones of inhibition and the diameters of these zones were measured in mm.

Microbial strains

The prepared plant extract was tested against a panel of test microorganisms. Five gram positive bacterial strains viz. *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC8750), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermis* (MTCC 3615), *Proteus vulgaris* (MTCC 744); two gram negative strains viz. *Escherichia coli* (MTCC 443) and *Enterococcus faecalis*(MTCC 439) were used in the study.

Standard antibiotics

For comparison of ZOI with the sample, standard antibiotics viz. Ciprofloxacin, Ampicillin (AP) 10mcg were employed.

Determination of nutrient content

The nutritive values of the plants were determined by the method described by Indrayan *et al.* (2005).

Statistical analysis

All the experiments were repeated triplicates and the results were expressed as mean ± standard error (SE).

Results

Qualitative phytochemical screening

Phytochemical screening tests for different extracts of *Ageratum conyzoides* revealed the presence of tannin, alkaloids, flavonoids, phenols, glycosides, cardiac glycosides, reducing sugar and saponin and marked the absence of phlobatannin, carotenoids, anthraquinone and free anthraquinone in all the samples as presented in Table 1.

Quantitative estimation of Phytochemicals

The quantitative estimation of phytochemicals revealed high phenol content in chloroform extract (Table 2) in terms of catechol equivalent (7.602±00 mg/g) and high flavonoid content in chloroform extract (Table 2) in terms of quercetin equivalent (6.476±4.738 mg/g) *Antimicrobial assay:*

Zone of inhibition on the test organisms for anti-

Table 1. Qualitative analysis of phytochemicals

Plant	<i>Ageratum conyzoides</i>	
Tannin	FeCl ₃	+
	Lead acetate	+
Phlobatanin		-
Alkaloids	Picric acid	+
	Mayer's reagent	-
	Dagendroff's reagent	+
Flavonoids		+
Phenols		+
Glycosides		+
Cardiac glycosides		+
Carotenoids		-
Anthraquinone		-
Free anthraquinone		-
Reducing sugars		+
Saponin		+

*"/+" indicates presence and "- " indicates absence.

microbial activity against the different extracts of *A. conyzoides* showed positive result only towards chloroform extract. Other three extracts were not active against any of the test organism. Chloroform extract was potent against four out of seven test organisms (Table 3) viz. 9 mm against *Staphylococcus aureus* and *Escherichia coli* and 7 mm against *Enterococcus faecali* and *Proteus vulgaris*.

Antioxidant assay

The antioxidant activity of the methanol, ethyl acetate, chloroform and hexane extract was measured by the ability to scavenge DPPH free radicals and ABTS free radicals. It was observed that ethyl acetate extract had higher DPPH radical scavenging activity (56.35±1.79) and hexane extract showed a higher ABTS radical scavenging activity (65.85±00)

Table 2. Total phenolic and flavonoid content of the plant extract

Extract	Phenol (mg catechol equivalent/gm dry material)	Flavonoid (mg quercetin equivalent/gm dry material)
Chloroform	7.602 ± 00	6.476 ± 4.738
Ethyl acetate	2.755 ± 0.18	5.237 ± 1.25
Hexane	2.392 ± 0.01	1.354 ± 0.04
Methanol	5.580 ± 0.75	5.36 ± 0.75

*values tabulated are average of triplicate of each category

Table 3. Antimicrobial activity of the plant extract

Extract of <i>Ageratum conyzoides</i>	Diameter of inhibition of zone (mm)						
	<i>Bacillus subtilis</i>	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermis</i>	<i>Escherichia coli</i>	<i>Enterococcus faecali</i>	<i>Proteus vulgaris</i>
Chloroform	-	-	9±1.2	-	9±1	7±0.4	7±0.8
Ethyl acetate	-	-	-	-	-	-	-
Hexane	-	-	-	-	-	-	-
Methanol	-	-	-	-	-	-	-

*'-/' indicates no activity. Zone of inhibition includes the diameter of well (in mm)

Table 4. Zone of Inhibition (ZOI) of standard antibiotics for antibacterial inhibition

	Diameter of inhibition of zone (mm)						
	<i>Bacillus subtilis</i>	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermis</i>	<i>Escherichia coli</i>	<i>Enterococcus faecali</i>	<i>Proteus vulgaris</i>
Ampicillin (AP) 10 mcg	-	-	-	-	10±1.2	10±0.1	12±2
Ciprofloxacin	-	-	32±0.5	-	22±1	28±4	36±6

as shown in Table 4 *Nutritive value:*

The nutritive value of *A.conyzoides* was found to be 352.359 cal/100 g (Table 5) with high percentage of carbohydrate (81.315) and least percentage of protein (0.0248).

Estimation of total Phenols and flavonoids content

From the standard curve equation of catechin; y =

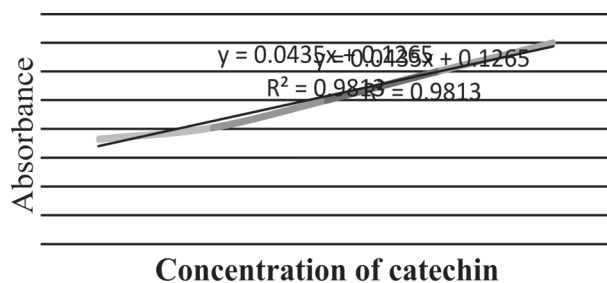


Fig. 1. Calibration curve of catechin

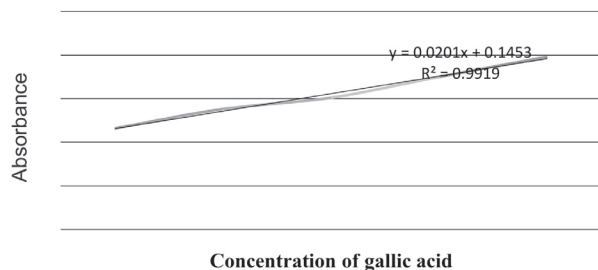


Fig. 2. Calibration curve of gallic acid

$0.043x+0.126$, $R^2 = 0.981$ (Fig. 1) and gallic acid; $y = 0.020x +0.145$, $R^2 = 0.991$ (Fig. 2).

Discussion

In the recent times, one of the growing areas is found to be the pharmaceutical industries due to rapid work performed to extract phytochemicals from different plants. The plants contain phytochemicals which possess antimicrobial as well as antioxidant activity that helps to fight against chronic diseases and other health issues and all these indicates the continuous relevance of the herb. The qualitative tests for Phytochemicals in *A. conyzoides* are summarized in Table 1 that confirmed the presence of tannin, alkaloids, flavonoids, phenols, glycosides, cardiac glycosides, reducing sugar and saponin and absence of anthraquinone, carotenoids and free anthraquinone. Amadi *et al.* (2012), found the presence of tannin, alkaloids, flavonoids, phenol, glycosides, saponin, HCN, steroids, coumarin, charomones, resins, carddenolides and terpenoids in different parts of the plant. Of the different parts investigated, leaves showed positive response towards every phytochemical test performed while the roots showed least presence of phytochemicals. Ukwe *et al.* (2010), in the phytochemical analysis of the extract and fractions revealed the presence of carbohydrates, reducing sugars, glycoside, alkaloids, flavonoids, proteins, saponins, tannins, fats and oil. Odeleye *et al.* (2014), also in their phytochemical screening of *A. conyzoides* indicated the presence of tannin, steroids,

Table 4. Antioxidant activities of the plant

Extract	Antioxidant activity (% inhibition in mg/ml)	
	DPPH radical scavenging activity	ABTS radical scavenging activity
Chloroform	53.31±00	57.45±0.9
Ethyl acetate	56.35±1.79	40.65±1.4
Hexane	42.09±0.54	65.85±2.0
Methanol	43.56±0.77	58.80±0.84
Ascorbic acid	93.77±0.014	96.11±0.002

*values tabulated are average of triplicate of each category

Table 5. Nutritive value of the plant

Plant	Percentage (%) of ash content	Percentage (%) of moisture content	Percentage (%) of fat content	Percentage (%) of protein content	Percentage (%) of carbohydrate content	Nutrient value cal/100gm
<i>Ageratum conyzoides</i>	5.16	10.5	3	0.0248	81.315	352.359

saponins, alkaloids, phenol, flavonoids and carbohydrate in ethanolic extract Kaur *et al.* (2018), in the phytochemical screening of various extracts of the plant revealed the presence of alkaloids, carbohydrates, amino acids, phytosterols, terpenoids and flavonoids. However, Agunbiade *et al.* (2012), Enerjiiofi and Isola (2019), Ndacnou *et al.* (2020) also revealed the presence of anthraquinones besides flavonoids, phenols, alkaloids, coumarins, saponins, tanins, sterols and glycosides in the phytochemical screening of the crude ethanol extract of *A. conyzoides*. Although some of the reports showed presence of anthraquinones, it was in trace amount and the variation in this study and those of other authors may be due to the use of different solvent or different plant part used.

In the investigation for phytochemical profile of ethanol extract of *A. conyzoides* by Harjanti *et al.* (2019), demonstrated the highest flavonoid (6.15 %w/v) and phenol (3.86%w/v) contents in comparison to the other plants used in their study. The flavonoid content in the chloroform extract of present study is more or less equal (6.476 ± 4.738 mg/g) to the ethanol extract but phenolic content is found to be higher (7.602 mg/g) in the chloroform extract in comparison to ethanol extract. The lowest phenolic (2.392 ± 0.01) and flavonoid content (1.354 ± 0.04) was recorded in hexane extract in the current study.

The chloroform extract also showed significant antimicrobial activity against four microbes out of seven, used for the experiment as presented in Table 3. Similar results were found in the investigation done by Dash *et al.* (2011). Odeleye *et al.* (2014) in their experiment for antimicrobial activity showed the zones of inhibitions (mm) of ethanol extracts of *A. conyzoides* on *Pseudomonas aeruginosa*, *E. coli*, *S. aureus* and *Shigelladysenteriae* at five different concentrations 5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml varied. Enerjiiofi and Isola (2019), in leaf extract of 300mg/ml gave high zone of inhibition of 24mm for *E. coli* against 9mm in present study. In the study conducted by Patil *et al.* (2010), zone of inhibition was also found against *B. subtilis* and *B. cereus* against essential oil extract of *A. conyzoides*. The variation may be due to the suitable environmental condition, different concentration, dose or different solvents used.

Further investigation conducted in the present study for DPPH and ABTS assay confirmed the antioxidant activity in *A. conyzoides*. Huang *et al.*

(2013), from their study concluded that the 95% ethanol extract of *A. conyzoides* showed good activities on both scavenging ABTS+ radical and reducing power, but low activity on scavenging DPPH radical. The present study also showed that the plant has got good amount of nutritive value.

Conclusion

The study shows that the plant can be used as a therapeutic agent as well as nutritive supplement along with the development of novel drugs. This type of phytochemical analysis is the first step towards understanding the nature of active principles in the medicinal plant which can be further helpful in discovering lead and active compounds after detailed study.

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