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Research Article

IN VITRO ANTI OXIDANT ACTIVITY OF CHROMATOGRAPHICALLY SEPARATED FRACTIONS FROM THE LEAVES OF *AGERATUM CONYZOIDES* L

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ABSTRACT

Synthetic anti oxidants are not safe for human health. It is often claimed that they may develop carcinoma in human body. Therefore, search for natural anti oxidants was going on and extended up to plant sources. Many medicinal plants are known having anti oxidant activity. *Aageratum conyzoides* Linn. is one such plant. In order to isolate anti oxidant compound (s) from the leaves of *A. conyzoides* L. the present study was undertaken. In isolation study silica gel G column chromatography of the powdered leaves of *A.conyzoides* L. was done when six fractions were separated. In vitro anti oxidant activity of these six fractions was measured by superoxide anion generation with help of xanthine-xanthine oxidase assay and with linoleic acid peroxidation assay as well as DPPH photometric assay. Results showed that fourth fraction had maximum anti oxidant activity. Inhibitory activities of xanthine oxidation, linoleic acid peroxidation and scavenging capacity of DPPH by the fourth fraction were respectively 96%, 97% and 96% whereas for other five fractions inhibitory activities were quite low.

Anti oxidant activity is known to be associated with compounds like total phenol, flavonoids, ascorbic acid and carotenoids. These compounds were estimated in the separated six fractions after chromatography of powdered leaves of *A. conyzoides* L. Results showed that fourth fraction had total phenol, flavonoids, ascorbic acid and carotenoids in the concentrations of 58 mg/mg dry wt, 88 mg/mg dry wt, 22 mg/g dry wt and 25 mg/g dry wt respectively. The amounts were significantly higher in comparison to that of other fractions. In vitro anti oxidant activity of the fourth fraction was, therefore, related with high amounts of total phenol, flavonoids, ascorbic acid and carotenoids. Present study indicated that the separated fourth fraction after silica gel G column chromatography of powdered leaves of *A.conyzoides* L. may be used as natural anti oxidant.

INTRODUCTION

A. conyzoides L. (family, Asteraceae) is a plant found in lowerand middle hill in Sikkim and Darjeeling up to 6000 ft. The plant has different names: Elame in Nepali, Namyew in Lepcha and Goat weed in English[1]. *A. conyzoides* L. grows commonly in the proximity of habitation, thrives in any garden soil and is very common in waste places and on ruined sites. Throughout the year the plant gives flower. Purple white flower appears.[2]

Medicinal value of *A. conyzoides* L. in treatment of a large number of human ailments is mentioned in Ayurveda, Charaka Samhita and Sushruta Samhita[2]. Leaves, root, stem and flower of this plant are widely utilized in traditional medicine. Leaves are styptic effective in healing of wounds, used in boils and prevent tetanus. Leaf juice is also used as eye lotion. The root juice has antibiotic property. The plant is boiled with oil and applied externally in rheumatism.[3]

Phytochemical screening showed that alkaloids, glycosides, flavonoids, ascorbic acid, phenol, essential oil, friedolin, caffeic acid, fumeric acid, kaempferol, sitosterol, stigmasterol and

unidentified esters are active components of *A.conyzoides* L.[4]

Modern researchers claimed that *A.conyzoides* L. has antibacterial[5] and wound healing effect[6]. It has neurological activity[7] and possesses gastro protective effect[8]. The plant acts as analgesic[9] and has effect on circulation[10]. It gives protection against gamma radiation[11]. The plant has anti tumor activity[12] and has allopathic effects[13]. Ita *et al.* (2009) demonstrated hepato protective activity of this plant[14].

Anti oxidant activity of plant *A.conyzoides* L. is known in literature[15-17]. We studied effect of season on anti oxidant activity *A.conyzoides* L. and noted that leaves of the plant had maximum in vitro anti oxidant activity in the months of July and August[18]. We intended to isolate active ingredient (s) from *A.conyzoides* L. leaves responsible for in vitro anti oxidant activity. In isolation process chromatographically separated fractioned were obtained. In this communication in vitro anti oxidant activity of chromatographically separated fractions of the leaves of A. *conyzoides* L. is reported.

MATERIALS AND METHODS

Collection of plant material

A. conyzoides L. leaves were collected in morning hours (9 - 10 AM) from the medicinal plants garden of the University of North Bengal, Dist. Darjeeling, WB, India randomly during July and August, 2016 as we have already seen that leaves of the plant had maximum in vitro anti oxidant activity in the months of July and August[18]. Leaves were authenticated by the experts of the department of Botany of the said University. A voucher specimen was kept in the department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences, Sikkim Manipal University, Gangtok, Sikkim, India for future reference.

Preparation of the test material

Collected leaves of *A.conyzoides* L. were shed dried and powdered. This powder was used as test material for extraction and isolation studies.

Extraction and Isolation

This was done by standard methods of isolation of chemicals from plant sources[19-20]. A sample of 50 g of powdered leaves of *A.conyzoides* L. was taken in 500 ml of ethanol and stirred by a rotary stirrer for 15 min at room temperature. The solvent was decanted and concentrated to 10 ml under reduced pressure using a rotary evaporator. This was then subjected to acid hydrolysis by refluxing with 10 ml N/10 hydrochloric acid at 100^0C for 10 mins to obtain a brown mass. The mass was extracted with 20 ml methanol for 10 mins. Material was centrifuged at 3000 rpm for 10 mins and the supernatant was subjected to column chromatography using silica gel G as adsorbent. Six bands were separated. Elution was done by 50% methanol-chloroform mixture. Six bands were separately collected and evaporated to dryness under reduced pressure using a rotary evaporator. Materials obtained were assayed for anti oxidant activity as well as total phenol, flavonoid, ascorbic acid and carotenoids content.

Antioxidant assays

Antioxidant activity of chromatographically separated fractions was assayed by superoxide anion generation by xanthine- xanthine oxidase assay[21], linoleic acid peroxidation assay[22]and by DPPH photometric assay[23].

Flavonoids content

Flavonoids content of chromatographically separated fractions was determined using Aluminum chloride colorimetric method[24].

Total phenolscontent

Total phenols content of chromatographically separated fractions was determined by Folin Ciocalteu reagent[25].

Ascorbic acid content

Ascorbic acid content of chromatographically separated fractions was determined by the method of Cakmak and Marschner[26].

Carotenoids content

Total carotenoids of chromatographically separated fractions were determined by the method of Jensen[27].

Chemicals

Chemicals required for the study were purchased from Loba Chem. Lab, Himedia Lab, India and from Merck, Germany.

Statistical Analysis

The statistical significance between antioxidant activity values of the extracts was evaluated with a Duncan's multiple range test (DMRT) at 5 % were considered to be statistically Significant[28].

RESULTS AND DISCUSSION

Results on antioxidant activity of chromatographically separated fractions of powdered leaves of *A.conyzoides* L. by superoxide anion generation by xanthine- xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay are given in Table. 1

Table 1: Inhibitory activity of xanthine oxidation and linoleic acid peroxidation and scavenging capacity of DPPH by chromatographically separated fractions of powdered leaves of *A. conyzoides* L.

Chromatographically separated fractions of powdered leaves of *A. conyzoides* L.	Xanthine oxidase (% inhibition)	Linoleic acid peroxidation (% inhibition)	DPPH (% inhibition)
Fraction ; 1	25	28	25
Fraction ; 2	36	35	32
Fraction ; 3	49	47	46
Fraction ; 4	96*	97*	96*
Fraction ; 5	47	40	42
Fraction ; 6	32	32	30
Quercetin	100	87	100

Concentration used : 100 µg/ ml based on our earlier experiment[18]. Results were a mean of triplicate experiments.*significant

It appears from the table that chromatographically separated all fractions of powdered leaves of *A.conyzoides* L. had more or less in vitro anti oxidant activity but maximum activity was with fourth fraction where 96%, 97% and 96% inhibitions in xanthine oxidase, linoleic acid peroxidation and DPPH respectively were noticed. Results were compared with quercetin, a synthetic anti oxidant, where inhibition in both xanthine oxidase and DPPH came 100%. For linoleic acid peroxidation inhibition was, however, 87%.

Total phenol, flavonoids, ascorbic acid and carotenoids contents of chromatographically separated fractions of powdered leaves of *A. conyzoides* L. are listed in Table -2. It appears from the table that maximum amounts of total phenol (58 mg/mg dry wt), flavonoids (88 mg/mg dry wt), ascorbic acid (22 mg/g dry wt) and carotenoids (25 mg/g dry wt) were present in fourth fraction of chromatographically separated fractions of powdered leaves of *A.conyzoides* L.

Fig.1: *Ageratum conyzoides* L.

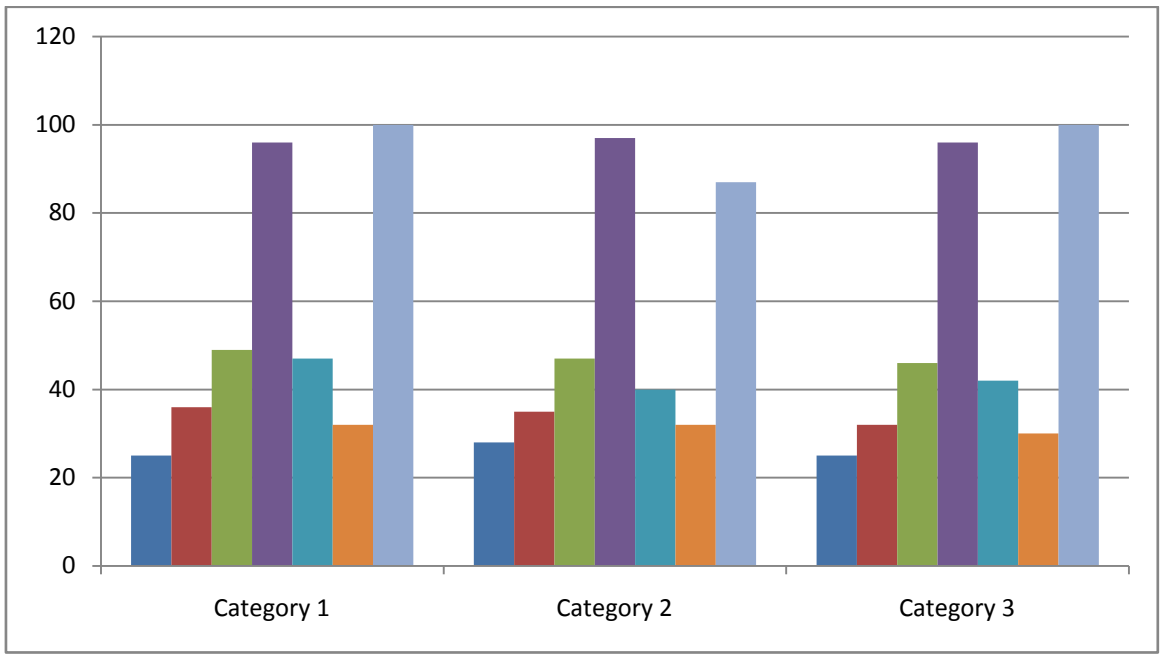
Fig. 2: Bands separation in Silica gel G column chromatography

Table 2: Total phenol, flavonoids, ascorbic acid and carotenoids content of chromatographically separated fractions of powdered leaves of *A. conyzoides* L.

Chromatographically separated fractions of powdered leaves of *A. conyzoides* L.	Total phenol content (mg/mg dry wt)	Total flavonoids content (mg/mg dry wt)	Ascorbic acid content (mg/g dry wt)	Carotenoids content (mg/g dry wt)
Fraction ; 1	10.2	10.2	2.3	7.2
Fraction ; 1	15.4	22.2	4.1	8.5
Fraction ; 3	20.8	31	6.9	11
Fraction ; 4	58*	88*	22*	25*
Fraction ; 5	15	30	7.3	9.9
Fraction ; 6	12	15	5.2	5.1

Results were a mean of triplicate experiments. *significant

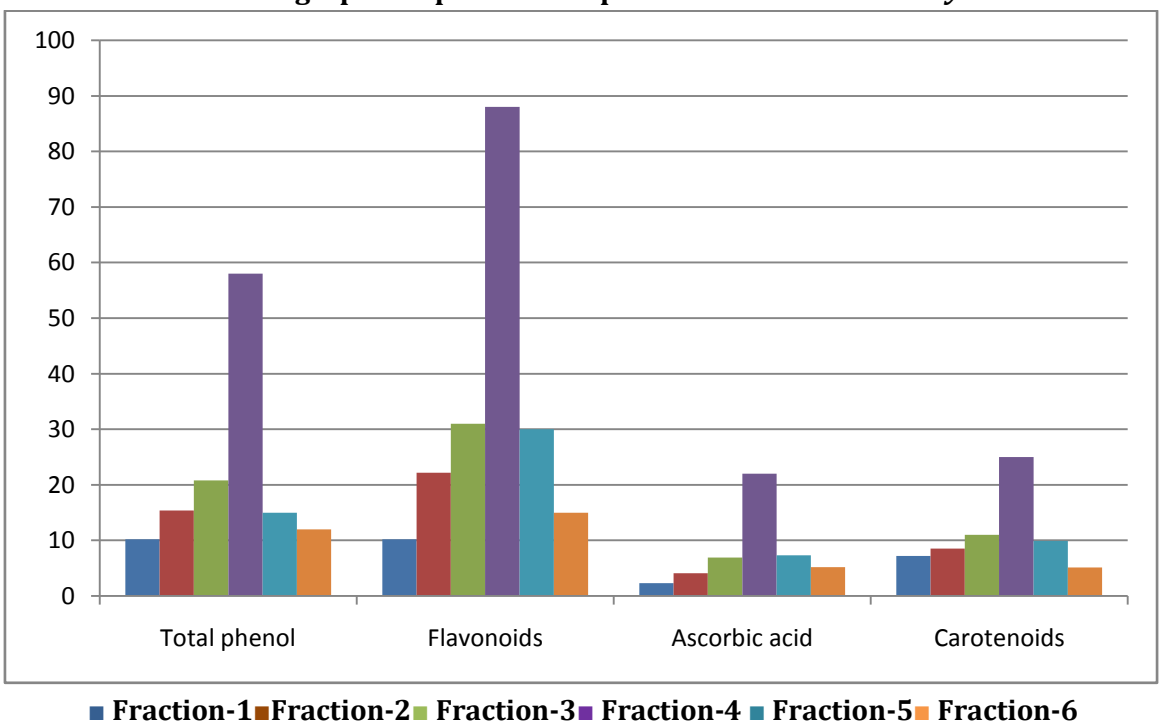
Figure 3: Showing % inhibition of xanthine oxidation, linoleic acid peroxidation and scavenging capacity of DPPH byseparated fractions of chromatographic experiment of powdered leaves of A. *conyzoides L.*



Category 1: Xanthine oxidase (% Inhibition) Category 2: Linoleic acid peroxidation (% Inhibition) Category 3: DPPH (% Inhibition)

Synthetic anti oxidants like butylated hydroxyanisole and butylated hydroxytoluene, though commercially available and commonly used in processed food, are not safe. Their toxicity is also matter of concern. It is often claimed that these synthetic anti oxidants have many side effect including carcinogenic activity[29]. Therefore, there are high demands for naturally occurring anti oxidants.It was known that leaves of *A.conyzoides* L. has anti oxidant activity. The present study was, therefore, undertaken to isolate anti oxidant compound from the leaves of *A.conyzoides* L. In isolation study chromatography was done. Six bands were separated. Anti oxidant activity of the separated six bands was studied separately. Fourth band showed maximum activity (Figure – 3).

Figure 4: Total phenol, flavonoids, ascorbic acid and carotenoids content of theseparated fractions after chromatographic experiment of powdered leaves of A*. conyzoides L.*



Anti oxidant activity of medicinal plant is mainly due to presence of phenolic compounds, flavonoids, ascorbic acid and carotenoids. These chemicals are responsible for multiple biological effects like free radical scavenging abilities, anti inflammatory and anti carcinogenic activities[30]. We, therefore, estimated amount of these anti oxidant compounds in the separated fractions obtained in chromatographic experiment with powdered leaves of A*. conyzoides L.* Results showed that amounts of phenolic compounds, flavonoids, ascorbic acid and carotenoids were maximum in the fourth fraction (Figure – 4).

CONCLUSION

The present study showed that the separated fourth fraction after silica gel G column chromatography of powdered leaves of *A.conyzoides* L. had maximum anti oxidant activity. The activity was associated with high amounts of phenolic compounds, flavonoids, ascorbic acid and carotenoids in the fraction. The fourth fraction, therefore, may be used as natural anti oxidant.

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