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DEVELOPMENT AND EVALUATION OF STABILITY OF GEL FORMULATION CONTAINING *Ageratum conyzoides* EXTRACT

SIRIKUNYA SAYOMPARK*, SOPITA TANGPIRIYAVAREE, YUPA KHONGPRIK and KHANITTA MEEPRADIT

Thai Traditional Medicine College, Rajamangala University of Technology Thanyaburi, Thanyaburi, Pathum Thani, Thailand
**E-mail: sirikunya_s@rmutt.ac.th*

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ABSTRACT

Ageratum conyzoides or 'Billy Goat Weed' is a weed distributed in many tropical and subtropical countries. It is widely utilized in traditional medicine by various cultures worldwide. The leaf, stem, root and flower of *A. conyzoides* was used to evaluate its chemical profile. Their extract exhibit a wide range of biological and pharmacological activities such as anti-inflammatory, anti-allergic, analgesic, antifungal, and anti-bacterial activities have been recorded. The objectives of the study were to evaluate the phytochemical screening tests of ethanolic extract *of A. conyzoides* and finally, the *A. conyzoides* extract was developed in Gel formulation. The *A. conyzoides* gel formulation was studied the stability at 45°C and heating/cooling condition, and then its appearance, viscosity, and pH value were determined. The presence phytochemical components of *A. conyzoides* extract include alkaloids, tannins, flavonoids and phenols. The AC-6 formulation was showed good physical properties: appearance, viscosity, and pH value by comparing it with marketed Diclofenac gel. The further study should be evaluated for anti-inflammatory and analgesic potency by animal paradigms, which may be helpful in developing novel new drugs.

Key words: *Ageratum conyzoides*, phytochemical screening, traditional medicine, gel formulation development

INTRODUCTION

Ageratum conyzoides or *'Billy Goat Weed'* is a weed distributed in many tropical and subtropical countries which belongs to Asteraceae family (Johnson, 1971). It is an erect, strong stem, unpleasant smell, herbaceous annual, 30-80 cm tall; stems are covered with fine white hairs, leaves are opposite, pubescent with long petioles and include glandular trichomes. The inflorescences contain 30-50 pink to purple flowers arranged as a corymb and are self-incompatible (Ming, 1999). The fruit is an achene with an aristate pappus and is easily dispersed by wind (Fagg & Clarkson, 2003). Seeds are positively photoblastic, and viability is often lost within 12 months (Marks & Nwachuku, 1986). The optimum germination temperature ranges from 20 to 25°C. The species has great morphological variation, and appears highly adaptable to different ecological conditions (Dan & Nhu, 1989).

It is widely utilized in traditional medicine by various cultures worldwide. In Southern of Thailand, the juice of the fresh plant and extract of the dried plant were used in the treatment of allergic rhinitis, sinusitis, and post-partum uterine haemorrhage. The dried powder is used to treat cuts and wounds. The powder absorbs the moisture of the disease and forms a layer that is removed after 1-2 days.

The leaf, stem, root and flower of *A. conyzoides* were used to evaluate its chemical profile. Their extract exhibit a wide range of biological and pharmacological activities. The active constituents of plants consist of alkaloids, tannins, flavonoids and phenols. The previous studies were identified alkaloids, mainly pirrolizidinic group such as 1, 2-desifropirrolizidinic and licopsamine (Ladeira *et al.,* 1987). The flower extract of *A. conyzoides* consist flavones such as ageconyfavones A, B, C and hexametoxyflavone (Horie *et al.,* 1993), which suggest that it may be a good constituents for herbal drug and product development. The whole plant is anti-inflammatory, anti-allergic, analgesic, anti-fungal (Vyas & Mulchadani, 1986; Borthakur &

* To whom correspondence should be addressed.

Baruah, 1987). Essential oil yield varies from 0.02–0.16% essential oil, which has anti-bacterial properties (Manandhar, 2002).

The previous study reported that the hydro-alcoholic of *A. conyzoides* extract at the dose of 250–500 mg/kg B.W. showed anti-inammatory activity in rat model of chronic inammation (Moura *et al.,* 2005). The ethanolic extract of *A. conyzoides* leaves dose 80–160 mg/200 g B.W. were able to significantly decrease the number of leukocytes, lymphocytes and udem volume, and decrease TNF alpha and MMP-9 levels on osteoarthritis rats induced by monosodium iodoacetate (Bahtiar *et al.,* 2017). Flavonoid glycoside compounds in the extracts of *A. conyzoides* leaves has an important role as an anti-inammatory compound (Awad *et al.,* 2013).

All of the useful, biological and pharmacological activities of *A. conyzoides* extract were appropriate to herbal drug formulation development. Delivery of drugs to the skin is an effective and targeted therapy for topical dermatological disorders and pain relief. The route of drug delivery has gained popularity because it avoids first-pass effects, gastrointestinal irritation, and metabolic degradation associated with oral administration (Prakash *et al.,* 2010). Topical gel formulations provide a suitable delivery system for herbal drugs because they are less greasy and can be easily removed from the skin. Percutaneous absorption involves the release of the herbal drug and permeates through skin to reach the target tissue (Patel *et al.,* 2011). Therefore, the topical gel formulation of *A. conyzoides* extract may be useful which also avoids the side effects associated with the oral therapy, which for anti-inflammation or pain relief.

The objectives of this study were to evaluate the phytochemical screening tests of ethanolic extract of *A. conyzoides* was collected in Nakhon Si Thammarat, Thailand, and finally, the *A. conyzoides* extract was developed in Gel formulation. The *A. conyzoides* gel formulation was studied the stability at 45°C and heating/cooling condition, and then its appearance, viscosity, and pH value were determined.

MATERIALS AND METHODS

Collection and preparation of plant material

The whole plants of *A. conyzoides* were collected in January, 2019 from the cultivated field in Nakhon Si Thammarat, Thailand. The plant specimen *of A. conyzoides* was identified by spot characters following by Tem Smitinand's Thai plant database, Department of National Parks, Wildlife and Plant Conservation, Thailand. The plant part were washed with tap water to remove the adhering dust particles, air dried under shade for three days and then oven dried at 50°C for 9 hr until constant weight (Prajapati *et al.,* 2013). The plants of *A. conyzoides* were grinded through mechanical grinder and converted into coarse powder and stored in container at room temperature until required.

Extraction of plant material

The plant powder of *A. conyzoides* was soaked in 95% ethanol. The plant powder in 95% ethanol was maintained at the ratio of 1:5 (w/v) for five days. The suspensions were stirred several times daily and then filtered using Whatman No.1 filter paper (Wuyep *et al.,* 2017). The residue was further soaked with the same volume of solvent two times. The combined solvent extracts were concentrated under reduce pressure through rotary evaporator at 40°C to obtain the crude extract and dried to constant weight in a desiccator. The crude extract was stored in deep freezer at -20°C until required.

Solubility test of plant extract

A series of solvents, including Methanol, 95% Ethanol, 80% Ethanol, 60% Ethanol, 50% Ethanol, Distilled water, Propylene glycol, Methyl salicylate, Coconut oil, and Sesame oil. The solubility of the extract was shaken in Vortex mixer and determined by increasing the concentrations 2-fold. The concentration was increased gradually until the extract was not soluble (Shah *et al.,* 2017).

Phytochemical screening test of plant extract

The plant extract of *A. conyzoides* were tested for the presence of alkaloids, tannins, flavonoids, phenols and terpenoids (Harborne, 1998; Trease & Evans, 1983). The qualitative results are expressed as "+" for the presence and "–" for the absence of phytochemicals (Morsy, 2014).

Test for alkaloids

Dragendorff's test, the extract (20 mg) was mixed in 10 mL of 2% Sulfuric acid and filtered. The filtrate was separated into portions, and then added a few drop of Dragendorff's reagent. Formation of orange or orange red precipitate indicates the presence of alkaloids by comparing it with Piperine.

Test for Tannins

The extract (20 mg) was dissolved in 6 ml of distilled water, and then added 10% Sodium chloride 1 mL. The mixture was filtered and separated 2 mL in test tubes. 1% gelatin solution containing Sodium chloride was added. Formation of white precipitate of Gelatin salt solution indicates the presence of tannins. The next separated test tube was added a few drops of Bromine water solution. Formation of pale

precipitate indicated the presence of condensed tannins. The next separated test tube was added a few drops of Lime water solution. The bluish-black colors formations showed the presence of hydrolysable tannins by comparing it with Tannic acid.

Test for flavonoids

The extract (10 mg) was mixed in ten drops of concentrated Hydrochloric acid followed by a small piece of magnesium were added. The mixture was diluted by distilled water 2-fold dilution, and then added 1 mL of Octyl alcohol, shaked vigorously. Formation of pink, reddish or brown color indicated the presence of flavonoids. Colors varying from orange to red indicated flavones, red to crimson indicated flavonoids, crimson to magenta indicated flavonones.

Test for phenols

Ferric chloride test, the extract (10 mg) of extract was mixed in 1 mL of absolute ethanol, and then adds a few drops of 10% ferric chloride solutions. The bluish color formations showed the presence of phenols.

Test for terpenoids

Salkowaski test, 10 mg of extract was mixed in 1 mL of chloroform, and then added 3 mL of concentrated sulfuric acid, carefully to form a layer. A reddish-brown coloration showed the presence of terpenoids.

Preparation of *A. conyzoides* Gels

Carbopol 934 was soaked in water for a period of 2 hr (Part A). In this experimental, the percentage of maximum extract that can be dissolved in based gel as 0.03. Then 0.03% w/w extract of *A. conyzoides* was dissolved in appropriate amounts of propylene glycol, glycerin and 95% ethanol (Part B). Triethanolamine (TEA) was mixed in Part B, and then phenoxyethanol was added with stirring. The mixture of part B, C and D were transferred to carbopol 934 container and agitated for additional 20 min. The dispersion was then allowed to hydrate and swell for 60 minutes, finally adjusted the pH with 98% TEA until the desired pH value was approximately reached (5.1–7). During pH adjustment, the mixture was stirred gently with a spatula until homogeneous gel was formed. All the samples were allowed to equilibrate for at least 24 hr at room temperature (Table 1).

Physical appearance

The developed gel formulations containing *A. conyzoides* extract were inspected visually for their color, homogeneity, and phase separation.

Heating cooling cycle

Six cycles between refrigerator temperature at 4°C and storage of 48 hr at 40°C were performed. Those formulations, which were stable at these temperature. The developed gel formulations after heating cooling cycle were subjected to Centrifugation test, Viscosity test and pH test (Komesmuneeborirak *et al.,* 2013).

Centrifugation test

The developed gel formulations were made to undergo centrifugation for 10 min at 3,000 rpm in a centrifuge. The stable formulations did not show any phase separation or turbidity (Kajornwongwattana *et al.,* 2016).

Viscosity test

Viscosity of developed gel formulations was determined by using viscometer. Each reading was taken after equilibrium of the sample at the end of two minutes. The measurement of Viscosity of each formulation was done in triplicate and average values are calculated, by comparing it with marketed Diclofenac gel.

Table 1. Gel formulation of *A. conyzoides* extract

Part	INCI Name	AC Gel Formulation (% w/w)					
		1	2	3	4	5	6
A	DI water	46.97	46.97	54.97	74.97	81.97	73.72
	Carbopol 934	2.00	1.00	0.76	1.00	1.00	1.50
B	AC extract	0.03	0.03	0.03	0.03	0.03	0.03
	Propylene glycol	1.00	1.00	1.00	1.00	1.00	1.00
	Glycerin	1.00	2.00	2.00	2.00	2.00	2.00
	95% Ethanol	47.00	47.00	39.24	20.00	12.00	20.00
C	Triethanolamine	1.00	1.00	1.00	1.00	1.00	0.75
D	Phenoxyethanol	1.00	1.00	1.00	1.00	1.00	1.00
	Total	100	100	100	100	100	100

pH test

The pH of developed gel formulations was determined using digital pH meter. The *A. conyzoides* gel (1 g) was dissolved in 100 mL distilled water. The measurement of pH of each formulation was done in triplicate and average values are calculated, by comparing it with marketed Diclofenac gel.

RESULTS AND DISCUSSION

The presence phytochemical components of *A. conyzoides* extract include alkaloids, tannins, flavonoids and phenols. Similarly, Ladeira (1987) found the alkaloids, mainly pirrolizidinic group such as 1,2-desifropirrolizidinic and licopsamine and Horie *et al*. (1993) found the the flavones such as ageconyfavones A, B, C and hexametoxyflavone. The *A. conyzoides* extract posse a remarkable anti-inflammatory, anti-allergic, analgesic, antifungal, and anti-bacterial properties (Table 2).

The average percentage yield of *A. conyzoides* Extract form 95% ethanol, five days are 13.70 at room temperature. The solubility of extracts in various solvents as shown (Table 3). The *A. conyzoides* extract was less soluble in water range concentration from 1 mg/mL to 8 mg/mL.

Gel formulations of *A. conyzoides* (AC-1 to AC-6) were prepared successfully by using different concentration. After Centrifugation test, it was observed that the characteristics of gel formulation AC-4, AC-5 and AC-6 did not show any phase separation or turbidity. The physical appearance, pH and viscosity were given in Table 4. Gel formulations AC-4, AC-5 and AC-6 were found neutral pH 5.17 to 6.82 and the viscosity of their formulations were 752.67 to 874.00 mPa.s.

From the above, the formulated gels were subjected for stability studies. No color fading or based gel formulation was observed for all prepared gels, by comparing it with marketed Diclofenac gel. After heating cooling cycle condition, the pH value of gel formulations of AC-4, AC-5 and AC-6 were found to be within the range of 5.36-6.41. Its pH value showed it could be appropriately used with the skin. It was contrasted with marketed Diclofenac gel, which decrease of pH value after heating cooling cycle condition. However, the skin irritation test will be reported in further work (Table 5).

Table 2. Phytochemical analysis of *A. conyzoides* extract

Chemical compounds	Test name	Observation	Result
Alkaloids	Dragendorff's test	Orange or Orange red ppt formed	+
Tannins	Gelatin solution test	White ppt formed	+
	Gelatin salt solution test	White ppt formed	+
	Bromine water test	Pale ppt	+
	Lime water test	bluish-black color formed	+
Flavonoids	Shinoda's test	Reddish or brown color formed	+
Phenols	Ferric chloride test	bluish color formed	+
Terpenoids	Salkowaski test	reddish-brown color formed	–

* ppt: precipitate.
** + the presence and - the absence of phytochemicals.

Table 3. The solubility of extract in various solvents

Solvent (w/v)	Solubility			
	1 mg/ml	2 mg/ml	4 mg/ml	8 mg/ml
Methanol	well soluble	well soluble	well soluble	well soluble
95% Ethanol	well soluble	well soluble	well soluble	soluble
80% Ethanol	soluble	soluble	soluble	soluble
60% Ethanol	soluble	soluble	soluble	soluble
50% Ethanol	soluble	soluble	soluble	soluble
DI Water	less soluble	less soluble	insoluble	insoluble
Propylene glycol	well soluble	well soluble	well soluble	soluble
Methyl Salicylate	well soluble	well soluble	well soluble	well soluble
Coconut Oil	well soluble	well soluble	less soluble	insoluble
Sesame Oil	well soluble	well soluble	less soluble	insoluble

Table 4. The Characteristics of *A. conyzoides* gel formulation

Characteristics	AC Gel Formulation		
	4	5	6
Color	Light green	Light green	Light green
Homogeneity	Transparent	Transparent	Transparent
Phase separation	Non-separation	Non-separation	Non-separation
pH	6.82±0.07	5.99±0.05	5.17±0.07
Viscosity (mPa.s)	807.67±1.69	752.67±1.15	874.00±2.65

Table 5. pH values of *A. conyzoides* gel formulation

Gel formulation	Heating cooling cycles condition		Sig.
	Before	After	
Based (B) gel			
B-4	6.28±0.07	6.39±0.05	> 0.05
B-5	6.01±0.08	6.42±0.07	> 0.05
B-6	5.53±0.13	5.90±0.03	> 0.05
A. conzoide (AC) gel			
AC-4	6.82±0.07	6.41±0.03	< 0.05
AC-5	5.99±0.05	6.52±0.12	< 0.05
AC-6	5.17±0.07	5.36±0.04	< 0.05
Marketed Diclofenac (MD) gel			
MD	6.43±0.03	5.82±0.02	< 0.05

Table 6. The Viscosity of *A. conyzoides* gel formulation

Gel formulation	Heating cooling cycles condition		Sig.
	Before	After	
Based (B) gel			
B-4	831.00±2.65	954.33±2.03	> 0.05
B-5	830.33±1.53	870.33±1.53	> 0.05
B-6	979.33±1.53	984.33±1.03a	> 0.05
A.conzoide (AC) gel			
AC-4	807.67±1.69	903.00±1.41	< 0.05
AC-5	752.67±1.15	868.33±1.44	< 0.05
AC-6	874.00±2.65	965.67±2.03	< 0.05
Marketed Diclofenac (MD) gel			
MD	488.33±1.75	353.33±1.50	< 0.05

The viscosity and spreadability of all gels was found to be same especially at ambient and 4°C, but at 40°C slight increase in viscosity was found the range of 868.33 mPa.s to 965.67 mPa.s. The viscosity of AC-6 formulation was shown less than AC-4 and AC-5, respectively. It was contrasted with marketed Diclofenac gel, which decrease of viscosity after heating cooling cycle condition (Table 6).

The AC-6 formulation showed good physical properties: appearance, viscosity, and pH value by comparing it with marketed Diclofenac gel. The further study should be evaluated for anti-inflammatory and analgesic potency by animal paradigms, which may be helpful in developing novel new drugs.

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