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PHYTOCHEMICAL & PHARMACOGNOSTIC STUDY OF *EUPATORIUM ADENOPHORUM* SPRENGE

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ABSTRACT

Eupatorium adenophorum Sprenge is a weed found at the hills of northern India, southern Nepal and Bhutan. It has many common names, including eupatory, Mexican devil, Sticky snakeroot and crofton weed. *Ageratina adenophora* is a synonym.Various species of *Eupatorium adenophorum* sprenge have been used in the traditional system of medicine across the world. *Eupatorium adenophorum* is accredited for diverse medicinal properties and finds therapeutic applications in traditional medicines asantiseptic, antimicrobial, blood coagulant, antipyretic, and analgesic. The total ash value is an indicative of total amount of inorganic material after complete incineration and the acid insoluble ash value is an indicative of silicate impurities, which might have arisen due to improper washing of drug. The results of phytochemical tests indicate the presence of glycosides, alkaloids, tannins, saponins and sugars. The result of present study will also serve as reference mono graph in the preparation of drugformulation.

KEYWORDS: *Eupatorium adenophorum*, Antiseptic, Antimicrobial, Glycosides, Alkaloids.

INTRODUCTION

Thousands of years have been known diseases and many ingenious methods have been followed for the relief of mankind. Every source of matter and life surrounding human being has been used in some form or other to treat diseases. The beginning of the history of many plants has been used for the cure of variousdiseases.

Eupatorium adenophorum Sprenge is a weed found at the hills of northern India, southern Nepal and Bhutan. It has many common names, including eupatory, Mexican devil, Sticky snakeroot and crofton weed. *Ageratina adenophora* is a synonym. The plant is primarily sourced for the diet of grass, which are one of the easily available in the sub-Himalayan region and have recognized for its therapeutic potential and utritional value. The plant of leaves is also used in cuts and wounds, also as Veterinary medicines in cuts and wounds,[2] analgesic,[3] and antibacterial. Chemically the plant has been found to be rich in flavonoides and cadenine derivatives; viz., α-cadinnine, naphthalene, 2,3,4,4a,5,6-hexahydro- 7-methyl–1-(1-methylethyl)-6- ol, salvigenin, eupifriedelinol, β-amirin, lupeol, stigma sterol, taraxasterol, isohexacosane, n-hexacosanoic acid and stigmastadienone.[1-2] Genus *Eupatorium* has been used traditionally in the treatment of emetic, diaphoretic, emmenagogue, cathartic, stimulant, tonic.[5]

Ethno medicinal significance

Various species of Eupatorium adenophorum sprenge have been used in the traditional system of medicine across the world. *E. adenophorum* is accredited for diverse medicinal properties and finds therapeutic applications in traditional medicines asantiseptic, antimicrobial, blood coagulant, antipyretic and analgesic. The leaf of plant is used to stop bleeding of cut and wounds, forming clots. A decoction of the leaf has been recommended to treat ulcers and jaundice diseases. Conventionally, decoction of leaves has been used on cut wounds to stopbleeding.[6]

MATERIALS AND METHODS

Plant material

The plant was collected in the month of February and March 2020 from the Forest of Ukhimath, Rudraprayag District, Uttarakhand India. The plant was identified and authenticated from Botanical Survey of India, Dehradun (Acc. no. 1127802).

Macroscopic and Microscopic analysis

The microscopy and macroscopy of the plant were studied as the method of Brain and Turner.[6] For the microscopical study, Transverse sections were prepared and stained.[7] The micro powder analysis and leaf constant was done as official method.[8-11]

Sample Name-*Eupatorium adenophorum* Sprenge.

Development of Standardization Parameters for *Eupatorium adenophorum* Sprenge

Study of Organoleptic characters

- Colour
- Odour
- Taste

Determination of Physiochemical parameters

- Moisturecontent
- Totalash
- Acid insolubleash
- Water solubleextractive
- Alcohol solubleextractive

Evaluation parmeters

- Powderfineness
- Bulkdensity
- Tapdensity
- Angle ofrepose
- Compressibility
- Hausner'sratio

Organoleptic Characters

The herbal plant is studied for organoleptic characters like color, odour and taste.

Physiochemical Analysis[12-18]

Determination of Loss on Drying (LOD)

10gsample (without preliminary drying) was weighed and placed on tarred evaporating dish. It was dried at 105˚C for five hours and at one hour interval until difference two successive weighing corresponded to not more than0.25%.

Determination of Total ash value

2 to 3g sample was weighed and placed in a tarred silica dish at a temperature not exceeding 450˚C until it was free from carbon, then cooled and weighed the sample. And calculate the percentage of total ash with reference to the air drieddrug.

Determination of Acid-insoluble ash value

The ash (total) obtained was boiled with dilute hydrochloric acid (25 ml in amount) for 5 minutes. An ashless filter paper was used to collect the insoluble matter obtained and ignited to constant weight after washing with hot water. Now the percentage of the acid insoluble ash easily be calculated by referencing with the air dried drug.

Determination of Water-soluble extractive value

5gm. of test sample was weighed and macerated with 100ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during 6hours and allowing standing for eighteen hrs. By taking precautions against the loss of solvent, it was filtered rapidly. In a tarred flat bottomed shallow dish 25 ml of it (filtrate) was taken for evaporation into dryness at 105°Cto constant weight and then the percentage of water soluble extractive was weighed by referencing with the air dried sample.

Determination of Alcohol-soluble extractive value

For the determination of alcohol soluble extractive the procedure for water soluble extractive was followed. But instead of Chloroform water, 90% ethanol was used.

Determination of Physical Characteristics Bulkdensity

Bulk density in actual is the ratio of given mass of powder and its bulk volume. An accurately weighed amount of powder sample was transferred to the graduated cylinder with the aid of a funnel to determine it. Now note the initial value. Ratio of weight of given mass powder vs volume occupied by it was calculated.

Bulkdensity=W/V0g/mlWhere,W=massofthepowder, V0=untapped volume

Tappeddensity

It was determined by transferring a known quantity (25g) of powder into a graduated cylinder and also tapping it for a specific number of times. Now note the initial value. The cylinder was now tapped for a continuous period (say 10-15 min). Now the density was calculated by finding ratio of mass of powder to the tappedvolume.

Tapped density =W/Vfg/ml
Where,W=massofthepowder,Vf=tappedvolume.

Compressibility index

It is the powder's propensity to be compressed. On the bassis of apparent bulk density and tapped density, we can find the percentage compressibility of the powder with the help of following formula-

Compressibility index = [(v0-vf) / v0] x 100, Or %Compressibility=[(tappeddensity–bulkdensity)] /tappeddensity]x100

Hausner'sratio

The powder's flow properties is indicated by this and the ratio hence obtained by tapped density and to the bulk density of the powder is called Haunser'sratio.

Hausner's ratio=Tappeddensity / bulkdensity

Angle of repose

It is defined as the internal angle between the surface of the pile of powder and the horizontal surface. A funnel fixed to a burette at a height of 4 cm was used to pass the powder. a graph paper was placed below the funnel on the table. Then, the height and the radius of the pile was measured. Now, angle of repose of the powder was calculated byformula-

Angle of repose= tan-1(h/r) Where, h=height of the pile, r = radius of the pile

Phytochemical Screening

Preliminary qualitative tests[19-25]

Preliminary qualitative phytochemical investigation was held for the extracts. Given below are the various tests andreagents-

Detection of Alkaloids

Before filtration all extracts were dissolved individually in dilute hydrochloric acid. After filtration the filtrates were used to test for the presence of alkaloids.

Mayer's test

Mayer's reagent few drops were added to above filtrates. Presence of White cream couloured Precipitate showed confirmatory test positive for alkaloids.

Wagner's test

Wagner's reagent few drops were added to above filtrates. Presence of Reddish brown coloured Precipitate showed confirmatory test positive for alkaloids.

Detection of Flavonoids

Lead acetate test

Few drops of lead acetate solution was poured into the extract. Formation of yellow color precipitate showed the flavonoids present in it.

Sulphuric acid test

Extracts were being treated with few drops of H2S04. Formation of orange color showed the flavonoids present in it.

Detection of Phenols: Ferric chloride test

Few drops of ferric chloride solution were poured into 10 mg of extracts. The presence of Phenol was confirmed once bluish black color formed.

Lead acetate test

10mg extract was being treated with few drops of lead acetate solution. Formation of yellow colour precipitate showed the Phenol present in it.

Detection of Tannins

Extract was heated on a water bath after mixing a small quantity with water. The mixture was then being filtered and after this ferric chloride added to the filtrate. Once dark green color formedit showed the tannins present init.

Detection of Proteins& Amino acids

Biuret test

Two drops of one percent Copper sulphate solution and equal volume of 40% NaOH solution added to 0.5 mg of extract. The appearance of violet colour showed the protein present init.

Ninhydrin test

Two drops of freshly prepared 0.2% Ninhydrin reagent was added and heated with about 0.5 mg of extract. The appearance of pink or purple colour showed the proteins, peptides or amino acids present init.

Detection of Triterpenoids

Salkowski's Test

Five mg of the extract of the leaves, flowers and seeds was mixed with two ml of chloroform and concentrated. Then, H_2SO_4 (3ml) was carefully added to form a layer. An appearance of reddishbrown colour in the inner face indicated thepresenceoftriterpenoids.

Detection of Carbohydrates

Anthrone test

0.5 ml of aqueous extract of drug was being added with 2ml of anthrone test solution. Once green or blue color indicated the presence of carbohydrate confirmed.

Fehling test

One ml of equal part of Fehling solution A and B was mixed with 2 ml of aqueous extract of drug and boiled the contents of test tube for few minutes untill a brick red ppt. is formed on itssurface.

Detection of Glycosides

Liebermann's Test

Whole aqueous plant crude extract was being added with 2 ml of acetic acid and 2 ml of chloroform. The entity of a glycine, steroidal part of glycosides are showed by the green color.

S. N.	Parameters	Value/ Inference
1.	**Organoleptic Characters**	
a)	Colour	Greenish
b)	Odour	Characteristic
c)	Taste	Astrigent
d)	Texture	Smooth
2.	**Physicochemical standards**	
a)	**Ash value (AV)**	(SEM) % w/w
*	Total ash value	17 ± 1.3
*	Acid insoluble ash value	15.80 ± 4.27
b)	**Extractive value (EV)**	(SEM) % w/w
*	Water soluble Ext. value	9.70 ± 6.6
*	Alcohol soluble Ext. value	7.66 ± 17.2
c)	**Loss of drying (LOD) / Moisturecontent**	% LOD (SEM) 0.16+8.5
3.	**Physical Characters of Powder**	(SEM)

a)	Bulk Density	0.44 ± 3.59
b)	Tapped Density	0.64 ± 2.47
c)	Carr's index	30.2 ± 0.52
d)	Hausner's ratio	1.43 ± 1.10
e)	Angle of Repose	34.4 ± 1.89

Killer-Kiliani Test

A solution of glacial acetic acid (4.0ml) with 1 drop of 2.0% $FeCl_3$ mixture was mixed with the 10 ml aqueous plant extract and 1 ml H_2SO_4 then,concentrated. The entity of cardiac steroidal glycosides was shown by a brown ring which is formed between thelayers.

Salkowski's Test

Five mg of the extract of the leaves, flowers and seeds was mixed with two ml of chloroform and concentrated. Then, H_2SO_4 (3ml) was carefully added to form a layer. An appearance of reddishbrown colour in the innerfaceindicated thepresence of triterpenoids/ Steroidal glycosides.

Procedure for Thin layer Chromatography, all extracts of crude drug[26-30]

1. The stationary phase was applied onto the plate and then allowed to dryand stabilize.
2. Withapencil,athinmarkismadeatthebottomoftheplatet oapplythesamplespots.
3. Then,samplessolutionsareappliedonthespotsmarkedo nthelineinequaldistances.
4. The mobile phase is poured into the TLC chamber to a leveled few centimeters above the chamber bottom. A moistened filter paper in the mobile phase is placed on the inner wall of the chamber to maintain equalhumidity.
5. Now, the plate prepared with sample spotting is placed in the TLC chamber so that the side of the plate with the sample line is facing the mobile phase. Then the chamber is closed with a lid.
6. The plate is then immersed, such that the sample spots are well above the level of mobile phase. Allow sufficient time for the development of spots. Then remove the plates and allow them to dry. The sample spots can now be seen in a suitable UV light chamber or any other methods as recommended for the saidsample.
7. Then calculate the Rf value of thesample.

RESULTS AND DISCUSSION

Phytochemical Screening

S. No.	Physiochemical Constituent	Chemical Test	EXTRACTS			
			PE	CE	AE	AqE
1	Alkaloids Test	Mayer's test	+	+	+	+
		Wagner's test	+	+	+	+
2	Flavonoids	Lead acetate Test	-	+	+	+
		H_2So_4 Test	+	+	+	-
3	Phenol	Ferric Chloride Test	+	+	+	+
		Lead acetate Test	+	+	+	+
4	Glycosides	Lieberman Reaction	+	+	+	-
		Killer-Killiani Test	+	+	+	+
		Salkowaski Test	+	+	+	-
5	Tannins	Ferric chloride Test	+	+	+	+
6	Protein and Amino Acid	Biuret Test	+	+	+	+
		Ninhydrine Test	+	+	-	+
7	Terpinoids	Salkowaski Test	+	+	+	+
7	Carbohydrates	Anthrone Test	+	+	+	+
		Fehling Test	+	+	+	+
8	Glycosides	Liberman's Test	+	+	+	+

Thin layer chromatography

Extracts	Solvent System			
	Alcohol	Chloroform	Pet ether	Rf value
Alcoholic Extract	100%	-	-	0 . 1 2
	-	100%	-	0.215
	-	-	100%	0.31
	50	50	-	0.2
	-	50	50	-
	50	-	50	0.63
	40	30	30	0.72

	30	40	30	0.31
	30	30	40	0.52
Chloroform Extract	100%	-	-	0.11
	-	100%	-	0.53
	-	-	100%	0.12
	50	50	-	0.91
	50	-	50	0.27
	-	50	50	0.33
	40	30	30	0.11
	30	40	30	0.11
	30	30	40	0.62
Pet. Ether Extract	100%	-	-	0.19
	-	100%	-	0.33
	-	-	100%	0.61
	50	50	-	0.92
	50	-	50	0.56
	-	50	50	0.88
	40	30	30	0.33
	30	40	30	0.62
	30	30	40	0.93
Aqueous Extract	100%	-	-	-
	-	100%	-	-
	-	-	100%	-
	50	50	-	-
	-	50	50	-
	50	-	50	-
	40	30	30	-
	30	40	30	-
	30	30	40	-

CONCLUSION

The total ash value is an indicative of total amount of inorganic material after complete incineration and the acid insoluble ash value is an indicative of silicate impurities, which might have arisen due to improper washing of drug. The loss on drying value obtained is an indicative of amount of moisture content present in the drug. The extractive values names water soluble and alcohol soluble indicates the amount of active constituent in given amount of plant material when extracted with respective solvent, values obtained supports the fact that drug is unexhausted which is contrary to lower extractivevalue.

The results of phytochemical tests indicate the presence of glycosides, alkaloids, tannins, saponins and sugars. From the heavy metal test it is concluded that this powder drug is free from heavy metals. Fromtheallabovevalues, itcanbeconclud edthatthequality of crudedrugis"GOOD".

From the present investigation various standardization parameters such as Physiochemical standards like total ash, acid insoluble ash, water & alcohol soluble extractive values, loss on drying, phyto- chemical analysis, flow properties and safety evaluation were carried out, it can be concluded that this crude drug contain all good characters of an ideal crude drug and it was found to be harmless, more effective andeconomic.

The sample showed satisfactory results, but the efficacy of the products can only be judged by doing the pharmacology of which is suggested as future scope of R&D. The study showed the contents of formulation present within the permissible limits as per WHO, all these investigations are not specified in the standard literature such as in pharmacopoeia, which could helpful in authentication of this powder. The result of present study will also serve as reference mono graph in the preparation of drugformulation.

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