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ORIGINAL ARTICLE

Evaluation of Phytochemicals in Leaf Part of *Atalantia monophylla* (Wild Lemon) and Bioinformatic approach for Evaluating its Medicinal Properties

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ABSTRACT
Plant synthesis thousands of chemical compounds which have medicinal properties. India is one of the major sources of medicinal herbs in the world. Atalantia monophylla is available with less population size in tropical climatic condition. Due to the presences of volatile oil, the species is more important in the terms of medicinal properties. Therefore, local sealers prescribe these for curing various ailments. The aim of the study is to evaluate the presence of phytochemicals and its medicinal properties. Phytochemical analysis of alkaloids, flavanoids, cardiacglycosides, protein, phenol, tannin, carbohydrates, coumarins, terpenoid, triterpenoids, quinones and saponins were recorded in various extracts of A. monophylla. The plant shows antioxidant properties. GC-MS and PASS result of the study revealed the presences of some biological compounds having medicinal properties such as phobic disorder treatment, antiseborrheic, antiosteoporotic, antieczematic etc.
Keywords: *Phytochemicals, Atalantia monophylla, antioxidant, GC-MS, PASS*

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INTRODUCTION

India is one of the 'mega diversity' countries of the world. It is ranked ninth in the world in terms of higher plant species richness. Nature has been a source of medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from natural sources, mainly based on their use in traditional medicine [1]. The plant based traditional medicine system continue to play an essential role in healthcare with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care. Plant products also have an important role in the health care system of the remaining 20%, who reside in developed countries [2]. Medicinal plants have been discovered and used as traditional medicine since prehistoric times.

Atalantia monopylla is a plant in the family Rutacea. Rutaceae family is also known as citrus family. The Rutaceae family consists of 140 genera and about 1300 species. They are woody climbers naturally found in tropical regions. They are commonly seen in the dry evergreen forests of South India from the coastal regions to about 600m altitude. The members of the genus *Atalantia* are important plants in the understory layer of the forests. They are commonly found in the disturbed areas such as road side, forest edges etc and also along the sides of streams and periphery of other water bodies where sufficient sunlight is available. Asiatic elephant use this plant as food. The fruits are used to make pickles [3]. They also have medicinal property. In addition to medicinal values various parts of *A. monophylla* is used for several other purposes. Juice from the berries is used for dyeing purpose. It is useful as a rootstock for breeding new cultivars of citrus Linn [4]. The Phytochemicals are chemical compounds produced by plant. They are naturally occurring plant chemicals (phyto means plant in Greek). They are produced by plant through primary or secondary

metabolism. They provide plants with color, odor and flavour [5]. *Atalantia monophylla* is used as an ethano botanical and folk medicine. This plant has been used in folk medicine for several purposes such as the treatment of chronic rheumatism and paralysis [6]. This plant used for medical treatments such as rheumatoid pain and glandular swelling. The roots possess antispasmodic, stimulant and antirhuematism property. The plants have been reported to have mosquitocidal activit [7]. The leaves are boiled with water and used externally for rheumatoid pain and glandular swelling. The leaves and bark of this plant are traditionally used in the treatment of vitiated kapha, vata, flatulance, hemiplegia, arthritis, skin disease, bacterial infections and malignancy [8]. The essential oil from the berries is reported to cure inflammations [9]. The herbal extract made from the leaves is used in hemiplegia due to the presence of an active ingredient compound liniment.

Antioxidants are compounds that inhibit oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organism. Antioxidants such as thiols or ascorbic acid terminate these chain reactions. To balance the oxidative state, plants and animals maintain complex system of overlapping antioxidants such as glutathione and enzymes (e.g catalase and superoxide dismutase), produced internally or the dietary antioxidants vitamin C and vitamin E [10]. The present study reveals the presences of some phytochemicals and antioxidant properties in *A. monophylla.*

Gas chromatography-mass spectrometry (GC MS) is an analytical method that combines the features of gas chromatography and mass spectrometry to identify different substances within a plant extract [11]. Applications of GC-MS include drug detection, fire investigation, and identification of unknown samples. It's used for identification of chemical composition in different plant extracts. PASS is known as Prediction of Activity Spectra for biologically active Substance. It is used for the identification of therapeutic uses of active compounds. It is a bioinformatics tool. The PASS (prediction of activity spectra for substances) software product, which predict more than 300 pharmacological effects and biochemical mechanisms on the basis of the structural formula of a substance, may be efficiently used to find new targets for some ligands and conversely, to reveal new ligands for some biological targets [12]. The present study reveals the presences of phytochemicals, antioxidant properties and medicinal properties of the biological compounds.

MATERIAL AND METHODS

Collection of plant material

The leaves of *Atalantia monophylla* is collected from Palakkad district. The collected plant leaves was washed with tap water, shade dried at room temperature and powdered using electric blender.

Sample preparation

30g of powered plant leaves was soaked in 150 ml of petroleum ether, butanol, ethanol, and water for 24 hours with shaking. This process was done in a sequential manner. After 24 hours the extract was filtered using Whatsmann No.1 filter paper. The container was weighed before and after filtration. The filtrate was evaporated and the crude extracts were weighed. The crude extract was stored in closed conical flask under refrigeration until use. The yield percentage was calculated. The extracts were then used for the phytochemicals analysis, verification of antioxidant activity, Gas chromatography-mass spectrometry (GC MS) and Prediction of Activity Spectra for biologically active Substance (PASS).

Preliminary qualitative phytochemical analysis

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in the various alcoholic and aqueous extracts of *Atalantia monophylla* leaf.

Lead acetate test:

1ml extract was taken and few drops of 10% lead acetate solution were added. Formation of yellow precipitate indicates the presences of flavanoids.

Keller Killiani test:

1ml extract was taken 1ml glacial acetic acid was added. 5% 1 drop ferric chloride was added, 3ml concentrated sulphuric acid carefully added to the side of the test tube. Formation of brown ring at interface is indicates presences of cardiac glycosides.

Braemers test:

2ml extract was taken and 2 or 3 drop of 5%ferric chloride solution was added. Formation of green-black or blue-back color indicates presences of tannins.

Keller Killiani test:

2ml extract was taken. Add 1 ml glacial acetic acid and concentrated sulphuric acid. Formation of blue color indicates presences of glycosides.

Test for coumarin

2ml extract was taken and 10% 3ml sodium hydroxide was added. Then add 3ml chloroform. Formation of yellow color indicates presents of coumarins.

Salkowski test:

1 mg extract was taken 0.2ml chloroform is added conc.H2SO4 carefully added to the side of the test tube. Formation of reddish brown ring indicates presence of terpenoids

Test for quinones

Take 1ml sulphuric acid with 1 ml plant extract shake well red color indicates quinones.

Hagers test:

Take a pinch of extract add few ml of diluted HCL. Stir well and filter the sample. To the filtrate 1 or 2 ml of Hagers reagent was added.

Antioxidant assay

Phosphomolybdenum assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto [13].

Procedure: 1ml of plant extract was dissolved in 1ml of DMSO. 100ml from the prepared sample was taken and 1ml of reagent solution was added to it and incubated in a boiling water bath at 95°c for 90 min. After 90 min, the absorbance of the solution was read at 695 nm. Ascorbic acid (10 mg 1ml DMSO) was used as standard. The phosphomolybdenum reduction extracts were reported in percentage.

Hydroxyl radical scavenging activity

Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acids moieties of cell membrane phospholipids and causes damage to cell [14].

Procedure: various concentrations of extract were taken and 1ml of iron EDTA solution, 0.5 ml of EDTA solution, 1ml of DMSO and 0.5 ml of ascorbic acid were added to it. The mixture was incubated in a boiling water bath at 80 to 90°c for 15 min. After incubation 1 ml of ice cold TCA and 3 ml of Nash reagent were added and the reaction mixture was incubated at room temperature for 15 min. The absorbance was read at 412nm. The %hydroxyl radical scavenging activity is calculated by following formula,

$$\% \text{ HRSA} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs sample}} \times 100$$

Where, HRSA is the Hydroxyl radical scavenging activity, Abs control is the absorbance of control and Abs sample is the absorbance of sample.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Ethanolic leaf extract of *Atalantia monophylla* was analyzed for the presence of different volatile compounds by Gas chromatography-Mass spectroscopy (GCMS) technique. GC-MS analyses of some of the potent volatile constituents present in the extracts were performed at "Tamil Nadu Agricultural University, Department of Nano Science and Technology", Coimbatore, Tamil Nadu and India. GC analysis of the extracts was performed using a GC-MS (Model; Thermo Trace GC Ultra) equipped with a DB-35MS fused silica capillary column and gas chromatograph interfaced to a Mass Selective Detector (MS-DSQ-II) with XCALIBUR software. For GC-MS detection, an electron ionization system with ionization energy of -70eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1ml/min and the sample injected was 1µl; Injector temperature was 250°C; Ion source temperature was 200°C. The oven temperature was programmed from 60° to 240°C at the rate of 5°C/min, held isothermal for 1minutes and finally raised to 240°C at 5°C/min. Interface temperature was kept at 250°C. The relative percentage of each extract constituent was expressed as percentage with peak area normalization.

Prediction of activity spectra for biological substance (PASS)

Prediction of Activity spectra for biological Substance is a software product designed as a tool for evaluating the general biological potential of an organic drug like molecule. PASS provides simultaneous prediction of many type of biological activity based on the structure of organic compounds. Thus, PASS can be used to estimate the biological activity profiles for virtual molecules, prior to their chemical synthesis and biological testing.

RESULTS

Phytochemical analysis

Presences of phytochemicals are identified. Petroleum benzene shows presences of cardiacglycosides and phenols, other secondary metabolites are absent in petroleum benzene. Butanol shows slight presences of saponins and proteins. Ethanolic extract shows high presences of coumarins and slight presences of

flavanoids, cardiacglycosides, phenols, terpenoids and carbohydrates. Aqueous extract shows presences of flavanoids and cardiacglycosides , phenols, coumarins, terpenoids and proteins (Table 1)

Antioxidant assay

Phosphomolybdneum assay

Ethanolic extract of A. monophylla shows maximum percentage of activity. Butanolic extract shows minimum percentage of activity. Moderate percentage of activity shows by petroleum benezene and water (Table 2).

Hydroxyl radical scavenging activity

Butanolic extract have highest percentage of activity. Ethanolic extract shows minimum percentage of activity. Petroleum benzene and water have moderate percentage of activity and the standard shows highest percentage of activity (Table3).

GC-MS ANALYSIS

The gas chromogram of *A. monophylla* confirmed the presences of various interesting compounds with different retention times us illustrated in figure 1. These compounds were identified through mass spectrometry attached with GC. The identified compounds and their retention time, molecular formula, molecular weight and peak area (%). Totally 43 compounds were detected in the ethanolic extract of leaf part of *A. monophylla*. Among, them the most prevailing major compounds were n Hexane (peak area 39.63%), propane, 2, 2-diethoxy (peak area: 30.56%), Allyl 2 ethyl butyrate (peak area 0.03%), tetraethyl silicate (peak area: 0.20%) etc (Table 4).

PASS

Prediction of activity spectra of biological active compounds in the *A. monophylla* revealed therapeutic uses of this plant. Out of the 43 compounds identified by GC-MS analysis 34 compounds shows medicinal properties. The medicinal properties of these compounds are given in table 5.

Table 1: Preliminary phytochemical analysis

Test	Petroleum benezene	Butanol	Ethanol	Water
Flavanoids	-	-	+	+++
Cardiac glycosides	+++	-	+	+++
Phenols	+	-	+	+
Saponins	-	+	-	-
Tannins	-	-	-	-
Quinones	-	-	-	-
Coumarins	-	-	+++	+
Terpenoids	-	-	+	+
Triterpenoids	-	-	-	-
Proteins	-	+	-	+
Carbohydrates	-	-	+	-
Alkaloids	-	-	-	-

Table 2: Phosphomolbdneum assay

SL No	Sample	Percentage of activity (%)
1	Petroleum benzene	33.33
2	Butanol	25.80
3	Ethanol	48.38
4	Water	41.17

Table 3: Hydroxyl radical scavenging activity

SL No	Sample	Percentage of activity (%)
1	Petroleum benzene	79.06
2	Butanol	88.37
3	Ethanol	55.81
4	Water	83.72
5	Standard	95.34

DISCUSSION

In the present study the medicinal plant species, *Atalantia monophylla* was investigated for its phytochemical and antioxidant properties. All plant parts synthesize some chemicals in themselves, which are metabolized during their physiological activities. Phytochemicals are non nutritive plant chemicals that have protective or disease preventive properties. Many phytochemicals are now studied extensively for their potential and treating some diseases

The present study carried out to screening phytochemicals from petroleum benzine, butanol, ethanol, and water extracts of leaf part of *A.monophylla* which revealed the rich variety of chemical constituents, such

as flavanoids, phenols, coumarins, cardiacglycosides, carbohydrates, saponins and terpenoids. Flavanoids and cardiac glycosides are highly present in water extract. Phenols, coumarins, terpenoids and proteins slightly present in water extract. Saponins, tannins, quinines, triterpenoids, carbohydrates and alkaloids are absent in water. Ethanolic extract of *A. monophylla* highly contain coumarins. Flavanoids, cardiac glycosides phenols, terpenoids and carbohydrates are slightly present in ethanol extract. Alkaloids, proteins, triterpenoids, quinines, tannins and saponins are absent in ethanol extract. Butanol extract slightly contain saponins and proteins. Other phytochemicals are absent in butanol extract. Cardiac glycosides are highly present in petroleum benzene extract. Phenols are slightly present in petroleum benzene extract. Other phytochemicals such as flavanoids, alkaloids, triterpenoids are absent in this extract.

Table 4: Identification of phytochemical compounds in the ethanolic extract of leaf part of *A. monophylla*- GC-MS result

SL. No	Name of the compound	Retention time (RT)	Peak area %
1	n Hexane	1.76	39.63
2	Propane, 2,2 -diethoxy	2.98	30.56
3	Allyl 2-ethyl butyrate	5.53	0.03
4	Tetraethyl silicate	7.20	0.20
5	2-Myristynoyl pantetheine	9.22	0.03
6	1-Dodecanol, 3,7,11trimethyl	12.99	0.07
7	Methotrexate	16.41	0.35
8	2-Bromotetradecanoic acid	21.90	0.09
9	2H- Indeno[1,2-b]furan-2- one	24.64	0.58
10	Tricyclo[20.8.0.0(7,16)]tria contane, 1(22), 7(16)- diepoxy	31.67	0.19
11	Hexadecanoic acid, butyl ester	34.83	3.92
12	Ethane, 1,1-diethoxy	2.56	9.94
13	Arginine	3.59	0.05
14	Octadecanoic acid, 3- hydroxy-, methyl ester	5.53	0.02
15	Hexadecane, 1,1- bis(dodecyloxy)	6.49	0.20
16	2-Myristynoyl pantetheine	7.95	0.08
17	E-9-methyl-8-tridecen-2- ol, acetate	8.77	0.01
18	Dasycarpidan-1-methanol, acetate (ester)	10.61	0.01
19	Hydrocinnamic acid, o- [1,2,3,4-tetrahydro- 2- naphthyl) methyl]-	11.69	0.10
20	D-Fractose, diethyl mercaptal, pantaacetate	12.23	0.04
21	Curan-17-oic acid, 19,20- dihydroxy-, methyl ester, (19S)	13.38	0.03
22	2,5-Octadecadiynoic acid, methyl ester	14.46	0.05
23	Aminoacetamide, N- methyl-N-[4-(1- pyrrolidinyl)-2-butynyl]	14.89	0.16
24	Bicyclo[3.2.1]oct-6-ene- 6,8-dimethanol, 1,7- dimethyl-4-isopropyl-, bis(3,5-dinitrobenzoate)	15.28	0.35
25	Carbamic acid	18.32	0.44
26	9,12,15-octadecatrienoic acid, 2 [(trimethylsilyl)oxy]-1- [[trimethylsilyl) oxy]methyl]ethyl ester,(Z,Z,Z)	19.27	0.01
27	Octadecanoic acid, 9,10- epoxy-18-(trimethysiloxy)-,methyl ester, cis	19.53	0.02
28	Digitoxin	20.80	0.03
29	8,8-Dimethyl- 3,3a,4,5,6,7,8,8b- octahydro-2H-indeno[1,2- b]furan-2-one	21.12	0.03
30	Neocurdione	22.54	0.21
31	Tert-Hexadecanethiol	23.12	0.26
32	9,10-Secocholesta- 5,7.10(19)-triene-1,3,- dio,25-- [(trimethylsilyl)oxy]-, (3a,5Z7E)	23.65	0.02
33	1H-Cycloprop[e]azulen-7- ol,	23.95	0.08
34	Salsoline	24.33	0.58
35	Phen-1,4-diol, 2,3- dimethyl-5-trifluoromethyl	28.93	0.40
36	estra-1,3,5(10)-trien-17a-ol	30.85	1.20
37	Tricyclo[20.8.0.0.(7,16)]tri acontane, 1(22), 7(16)- diepoxy	31.67	0.19
38	1-Heptatriacotanol	32.11	0.01
39	Phytol	33.57	2.75
40	Hexadecanoic acid, butyl ester	34.83	3.92
41	9,10-secocholesta- 5,7,10(10)-triene-3,24,25- triol,(3a,5Z,7E)	36.07	0.23
42	Butyl 9,12,15- octadecatrienoate	31.81	3.96
43	Octadecanoic acid, butyl ester	38.22	0.54

Table 5: Medicinal properties of bioactive compounds by PASS prediction

S No	Name of the compound	Activity
1	Ethane, 1,1-diethoxy	Antiinflammatory Antiseborrheic Phobic disorder treatment Leukopoiesis stimulant
2	Propane, 2,2-diethoxy	Antiosteoporotic Antiobesity Antihypertensive Phobic disorders treatment Antiseborrheic Leukopoiesis stimulant Bone disease treatment
3	Arginine	Mucositis treatment Phobic disorders treatment Antiseborrheic Alopecia treatment Preneoplastic conditions treatment Acidifying agent gastric
4	Octadecanoic acid, 3-hydroxy-, methyl ester	Lipid metabolism regulator Phobic disorders treatment Vasodilator, peripheral Antiinflammatory, intestinal Mucositis treatment Antieczematic Eye irritation, inactive Leukopoiesis stimulant Platelet aggregation stimulant
5	Allyl 2-et.yl butyrate	Eye irritation, inactive Skin irritation, inactive Antieczematic Phobic disorders treatment Anesthetic general Respiratory analeptic
6	Hexadecane, 1,1-bis(dodecyloxy)	Phobic disorders treatment Sclerosant Leukopoiesis stimulant
7	Tetraethyl silicate	Dermatologic Phobic disorders treatment Antiseborrheic
8	Myristynoyl pantetheine	Lipid metabolism regulator Antipsoriatic Growth stimulant
9	E-9-Methyl-8-tridecen-2-ol, acetate	Mucomembranous protector Lipid metabolism regulator Antiviral (Rhinovirus) Antiinflammatory Antisecretoric Antieczematic Antithrombotic
10	Hydrocinnamic acid,o-[(1,2,3,4-tetrahydro- 2-naphthyl)methyl]	Phobic disorders treatment Acute neurologic disorders treatment
11	D-Fractose, diethyl mercaptal, pantaacetate	Lipid metabolism regulator Restenosis treatment Antiseborrheic
12	1-Dodecanol, 3,7,11-trimethyl	Phobic disorders treatment Antiseborrheic Eye irritation, inactive Antisecretoric Anesthetic general
13	Curan-17-oic acid, 19,20-dihydroxy-, methyl ester, (19S)	Respiratory analeptic Cardiovascular analeptic

14	2,5-Octadecadiynoic acid, methyl ester	Phobic disorders treatment Antiinflammatory Antieczematic
15	Aminoacetamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]	Spasmolytic, urinary
16	Bicyclo[3.2.1]oct-6-ene-6,8-dimethanol, 1,7-dimethyl-4-isopropyl-, bis(3,5- dinitrobenzoate	Antieczematic
17	Methotrexate	Antineoplastic Antiviral (Poxvirus Antimetabolite
18	9,12,15-octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[trimethylsilyl)oxy]methyl]ethyl ester,(Z,Z,Z)-	Antieczematic Antineoplastic
19	Octadecanoic acid, 9,10-epoxy-18-(trimethysiloxy)-,methyl ester, cis	Angiogenesis stimulant Antineoplastic
20	Digitoxin	Anesthetic general Proliferative diseases treatment Antineoplastic Antiprotozoal (Leishmania) Immunosuppressant Antineoplastic (lung cancer) Respiratory analeptic
21	8,8-Dimethyl-3,3a,4,5,6,7,8,8b-octahydro- 2H-indeno[1,2-b]furan-2-one	Antieczematic Antineoplastic
22	2-Bromotetradecanoic acid	Phobic disorders treatment Eye irritation, inactive
23	Neocurdione	Antieczematic Dermatologic
24	9,10-Secocholesta-5,7.10(19)-triene-1,3,- dio,25--[(trimethylsilyl)oxy]-, (3a,5Z7E)-	Dermatologic Antieczematic Bone diseases treatment Antiosteoporotic Antipsoriatic Antineoplastic Respiratory analeptic Proliferative diseases treatment Adenomatous polyposis treatment Immunosuppressant Antipruritic Hyperparathyroidism treatment
25	Salsoline	Antidyskinetic Skeletal muscle relaxant
26	phen-1,4-diol, 2,3-dimethyl-5- trifluoromethyl	Antiischemic, cerebral Phobic disorders treatment Antidyskinetic Antiseborrheic
27	estra-1,3,5(10)-trien-17a-ol	Antiseborrheic Alopecia treatment Antisecretoric Menopausal disorders treatment Bone diseases treatment Antihypercholest Antiosteoporotic Adenomatous polyposis treatment Prostate disorders treatment erolemic
28	Tricyclo[20.8.0.0.(7,16)]triacontane, 1(22), 7(16)-diepoxy	Antiseborrheic Alopecia treatment Antisecretoric Menopausal disorders treatment Antihypercholesterolemic Bone diseases treatment Antiosteoporotic Mucomembranous protector

		Adenomatous polyposis treatment Anesthetic general Prostate disorders treatment
29	1-Heptatriacotanol	Phobic disorders treatment Cardiovascular analeptic Vasoprotector Eye irritation, inactive Antiseborrheic Antieczematic Antihypoxic Skin irritation, inactive Mucomembranous protector Preneoplastic conditions treatment Mucositis treatment Anesthetic general Cytoprotectant Antineurotic
30	Phytol	Phobic disorders treatment Mucomembranous protector Antiulcerative Antiviral (Rhinovirus)
31	Hexadecanoic acid, butyl ester	Phobic disorders treatment Eye irritation, inactive Antieczematic Preneoplastic conditions treatment Mucomembranous protector Antihypoxic Skin irritation, inactive Anesthetic general Vasoprotector Antiseborrheic Antisecretoric
32	9,10-secocholesta-5,7,10(10)-triene- 3,24,25-triol,(3a,5Z,7E	Antieczematic Antineoplastic
33	Butyl 9,12,15-octadecatrienoate	Antieczematic Antihypercholesterolemic Phobic disorders treatment Eye irritation, inactive Antiinflammatory Antisecretoric Antithrombotic Vasoprotector Antiulcerative Antipruritic Skin irritation, inactive
34	Octadecanoic acid, butyl ester	Phobic disorders treatment Eye irritation, inactive Antieczematic Preneoplastic conditions treatment Mucomembranous protector Antihypoxic Mucositis treatment Vasoprotector Antiseborrheic Antisecretoric

CONCLUSION

It concluded that the study species *A. monophylla* contains considerable amount of secondary metabolites and the species also have antioxidant properties. The biological compounds present in the plant processes medicinal properties. That's why this plant used for several medicinal purposes.

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