INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN: 2277-7881; IMPACT FACTOR – 6.014; IC VALUE:5.16; ISI VALUE:2.286
VOLUME 8, ISSUE 8(6), AUGUST 2019

PHYTOCHEMICAL AND MEDICINAL INVESTIGATION OF WEEDS ECLIPTA PROSTRATA AND PENDALIUM MUREX

Khobragade Karuna H.
Department of Zoology
KET'S Vaze College of Arts, Science and Commerce Mulund (E)
Mumbai

Ragde Vinod
Department of Zoology
KET'S Vaze College of Arts, Science and Commerce Mulund (E)
Mumbai

Abstract

Weeds are unwanted and undesirable plants which interfere with the utilization of land and water resources. This green vegetation is also flourished in Aquatic systems, forestry, Industrial sites, Roadside, Railway lines, landscapes water tanks and waterways. Information about medical aspects of these weeds are been gathered from local people, Vendor and experts from Ayurvedic field. Basically, Medicinal importance of plants is due to the presence of some specific substances like Primary metabolites which enhance growth and metabolism within plants and secondary metabolites are produced from primary metabolites they play an important role in different metabolic activities of living organisms such as attracting pollinators and chemical defense against micro-organisms, insects and higher predators. Many such natural products have been used in Industrial products, agricultural chemicals, Pharmaceuticals and as food additives. Some of the secondary metabolites are potentials drugs, essential oils, alkaloids, antibiotics, cardiac glycosides, coumarone, lignin's, resins, sterols, Sapogenins, Tannins, Volatile oils and Insecticides.

Two such weeds Eclipta prostrate and Pendalium murex were studied to detect and identify which secondary metabolites are present in these plants so Flavonoids and sterols are some of the secondary metabolites which are found in these plants which are further Identified, extracted, Isolated through the Thin layer chromatography, then Quantitatively estimated followed by comparative study of these two species that which part of the plant yield more metabolites as compared to the other species. It is being observed that Flavonoids are maximum (6.30mg/g of d.w) in the leaves of Eclipta prostrata and minimum (3.30mg/g d.ws) in the fruit of Pendalium murex and the sterol content is maximum in fruits of Eclipta prostrata (3.04mg/g d.w)and minimum in the roots of Pendalium murex (1.73mg/g d.w)

Keywords: weeds, Ayurved, medicinal aspect, Flavonoid, Quercetin, Kaempferol, Sterol β-Sitosterol, Stigmasterol, Phytosterol

Introduction

Weeds of arid area are a good source of phytochemically important compounds. The plants for study were collected from Haryana. The region of the Northwest part of Indian in the states of Rajasthan and Haryana is the area between the Indus river in the west and the Aravalli mountain in the east are described as desert soil the local inhabitants as well as in an indigenous system of medicine utilizes the medicinal plants of the region.

In the last few decades, there is a growing demand of medicinal plants by pharmaceutical companies. This increasing demand if properly utilize can help in village economy as well as will open new avenues of employment. The per hectare income generated from growing medicinal plants is much more than any other crops. On the other hand, the land which is not suitable for crop cultivation can be utilized for the cultivation of medicinal plants, which is suitable for that habitat.

India has a vast and inexhaustible resource of drugs of plant origin. Several important medicinal and Aromatic plants prescribed by Vaidya and Hakim have carefully investigated from every point of view.

Mathur, S.K., Kapoor, BBS and, Nag, T.N. (1988), Purohit, G.R., (1977); Acharya, J.(1999); Harsh, M.L. and Maheshwari, A.(2000); Kapoor, BBS and Ritu (2001); Harsh, G.(2002); Kapoor ,BBS and Kalla and Nishi Prabha(2003).

Ethnobotanical aspect

Weeds are an important source of medicines. These are used by human beings for the treatment of many diseases from ancient times. The traditional Medicinal plants through out the world played an important role in the development of a new Herbal drug. India is known for some of the medicinal plants which are in great demand all over the world. Almost 540 species are utilized by Pharmaceutical formulations(Kapoor and Mitra 1979).Medicinal importance of plants are due to the presence of specific substances like primary metabolites and secondary metabolites (Luckiner., November 1977).

UNESCO (1960) has published a survey report with details of the world medicinal Plant growing in different arid zone belonging to different families which are most adapted to dry condition for their growth and production of secondary metabolites in plants under water supply. They occur in higher plants but are uncommon in cryptogams. They impart colourto flowers and fruits. Correlation between flower colour and attraction of insects for pollination is well known, however, their occurrence is not restricted to flowers but include all parts of the plant.

Phytochemical aspect

Some secondary metabolites are Flavonoids and Steroids

Flavonoids: They are polyphenolic compounds possessing 15carbon atoms, 2 benzene ring joined by linear three carbon atom. Some Flavonoids are of Pathological significance whereas others are physiologically important to animals. They show therapeutic hemorrhagic condition (PonVelayutham., AnandhBabu and DongminLui, 2009). They also show protection against a nuclear hazard(YahyapourR,Shabeeb D et.al 2018).

Steroids: Theyare the most important group of secondary metabolites. Steroids occur abundantly in the vegetable world than in animals and therefore plant steroids are considered as a precursor for hormone synthesis. The sapogenins, diosgenin which is abundantly available, is of particular importance for the production of sex hormones. The phytosterols are ubiquitous in higher plants and probably also in plant tissue culture. Indeed it is probably that they are essential components of many cellular membranes.

The steroids attached with sugar are very common in plants one class of these substances are known as saponins. The steroidal part of these saponins called sapogenins.

Sapogenins are widely used in the field of medicines, as they are the main precursor of many medicinally useful steroids hormones. Chemically Steroid represents a non-saponifiable fraction of lipids extracted in fat solvents.They bear cyclopentano-perhydro-phenanthrene nucleus in their molecular structure. The steroid consists of 4 rings A, B, C, D. A, B and C rings are six-membered and D is 5 membered rings. If the compound has one hydroxyl group and no carboxyl group it is a sterol. If it has one or more carbonyl group or carboxyl group then it is a steroid.And we know that steroid is the precursor of hormone synthesis.

Research and Methodology

Some of the weeds, are used by local people for Medication. A research was conducted by interviewing the Hakim's,Vaidhya's and Ayurvedic expert of that area. And the dosage and the

Mixture was studied and how it is helpfulto mankind and curing the ailment, though natural with no side effects.This investigation had thus inspired me to find out which are the natural substances present in the weeds, so this research was conducted.

Collection of plants: Plants were collected from Rohtak District State Haryana, which is above 220 metres above sea level. Two weed species Eclipta prostrata and Pendalium murex were taken for study.

Eclipta prostrata
Family: Asteraceae
Local Name: Bhringaraaja, Bhringa.
Habitat: Throughout India up to 2000 m on the hill.
Characteristics: The plant found the whole of the year and measures about 50 cm in length. The branches are green, shiny, with some blackish shade. The branches have small white hairs. Leaves are 2-6 cm in length and 2-3 cm in breadth. On crushing the leaves a blackish green fluid squeeze out, which turn out black after some time.
Flowering and Fruiting: Almost throughout the year.

Pendalium murex:
Family: Pedaliaceae
Local Name: Ghokhru.
Habitat: North-Western India.
Characteristics: The plant grows in the rainy season and obtains a height of 50 cm. Its branches remain bent towards the ground. The flowers are small-sized and yellow. Its fruit has four spines.
Flowering and Fruiting: October and November

Preparation of plant Material
Leaves of Eclipta and Pendalium were collected from the study area, thoroughly washed with tap water wiped air-dried cut into small pieces. Further dried at 100 % for 15 min to inactivate enzyme then further dried at 60ºC till constant weight is achieved, then powdered.

Extraction
Flavonoids
The dried samples were separated soxhlet extracted(Subramanium and Nagrajan,1969) in 80 % Ethanol (100ml/gm dry weight) on a water bath for 24 hrs. Each of the extracts was concentrated and the concentrate wasre-extracted into petroleum ether (40-600C: fraction 1) ethyl ester (fraction 2) ethyl acetate (fraction 3) in succession. Each of the steps was repeated 3 times to ensure complete extraction in each case.Fraction 1: was rejected due to its being rich in fatty substances; Fraction 2: was analysed for free flavonoids; Fraction 3: was analysed for bound flavonoids
Fraction 3 of each test sample was hydrolysed with 7 % sulphuric acid (mg/gm residue) for two hours.This mixture was filtered extracted with ethyl acetate in separating funnel. The ethyl acetate layer (upper) was washed with distilled water to neutrality. With the help of thin layer chromatography fraction 2 & 3 were assessed for flavonoids.

Chromatography

Thin-layer chromatography for flavonoids.

Ethyl ether and ethyl acetate fraction from each of the following test sample were uploaded standard reference compound(Apigenin, Kaempferol, Luteolin, Quercetin and Vitexin) using solvent mixture of n-butanol, acetic acid and water(4:1:5; upper layer).

Several other solvent mixtures such as ethyl acetate saturated with water, acetic acid (6:4) forestall system(acetic acid, conc.HCL and water; 10:3:30) were also tried. The solvent mixture of n-butanol, acetic acid, water(4:1:5) gave the best result in all the cases examined. The developed plates were air-dried and visualized under UV light which showed two fluorescent spots in both the fractions 2 and 3 in all the instances co-including with those of the standard sample of Quercetin (blue Rf 0.82) and Kaempferol (bright yellowish blue, Rf 0.93). The plates were placed in a chamber Saturated with ammonia vapours to observe the colour of the spots (Quercetin, Deep yellow; Kaempferol, light yellow). On spraying the developed [plates with 5% ethanolic $FeCl_3$ solution which also showed only one spot(in both the fraction 2 and 3) in which fraction was considered with that of the reference of Quercetin (bluish-grey) and fraction 3 with Kaempferol brownish). The Rf values were calculated on an average of the 5 replicate.

Preparative thin-layer chromatography(PLC)

The extract of both the fraction (2and3) was applied on separate plates and the developed plates were air-dried and visualized under UV light. Each of the fluorescent spot coinciding with those of standard reference compounds of Quercetin and Kaempferol were marked. The marked spot was scrapped and collected separately along with the silica gel and eluted with ethanol. Each of the elute was crystallized with chloroform.

The compound thus isolated, were subjected to colourimetric (quantitative estimation)m.pt,(Melting point apparatus, Toshniwal, India), UV maxima on a spectrophotometer on infrared spectral studies.

Quantitative estimation

Quantitative estimation flavonoids were carried out colourimetrically following the method of Kariyone et al. (1953) and Naghhski et al. (1975) in case of quercetin; and Mebry et al. (1970) in case of Kaempferol. Stock solution (25micro gm/ml) of quercetin and Kempferol were separately prepared by dissolving the authentic sample in methanol. Six conc.,(25 micro gm/ml-150 micro gm/ml) of each of the standard samples were spotted on silica gel coated and activated plates. Separate plates of each of the conc. Of quercetin and Kempferol were used and these chromatograms were developed in the same

solvent system as used for the quantitative method (n-butanol; acetic acid; water,4:1:5, upper layer). Such developed chromatograms were air-dried and visualized under UVlight. The fluorescent is marked and collected along with the adsorbent in separate test tubes the mixture shaken vigorously, centrifuged and supernatants collected separately. The volume of the replicates was made up to 10 ml by adding spectroscopic methanol. To each of these sample 3ml of 0.1mole $AlCl_3$ was added, stoppered tightly and the mixture was shaken vigorously. Such tubes were kept at room for 20min. Five such replicates were prepared in each case and optical density is measured using spectronoic -20 colourimeter set at 440 nm for quercetin and 423 nm for kaempferol against a blank (10ml spectroscopic methanol+3ml 0.1 mole $AlCl_3$.

Table 1.Flavonoid contents of Eclipta prostrata and Pendalium murex

Plants	Plants parts	Quercetin	Kaempferol	Total
Eclipta prostrata	Leaves	1.10mg/gm.d.w	5.20mg/gm.d.w	6.30mg/gm.d.w
Pendalium murex	Fruits	0.10mg/gm.d.w	3.20mg/gm.d.w	3.30mg/gm.d.w

Sterols

Dried plant parts in powdered form were taken and used for extraction of sterols. Each dried sample was hydrolyzed with 30 % HCl 4 hours on a Water Bath. The hydrolyzed test samples were filtered and washed with D/W till the filtrate attain PH 7.0. Test samples so obtained were dried at 60°C for 8 hours and soxhlet extracted in Benzene (200 ml) 24 hours separately. Each of the Benzene extracts of the various test samples was dried in Vacuo and taken up in chloroform for further analysis. With the help of thin layer chromatography two sterols ß Sitosterol (Rf0.52) and stigmasterol (Rf 0.49) were observed in the plant samples.

Thin-layer chromatography

Each of the crude extracts applied separately on the silica gel G coated and activated thin glass plate along with a standard reference sample of sterols (β-Sitosterol, Campesterol, cholesterol, Lanosterol, and Stigmasterol). The plates were developed in an organic solvent which is a mixture of benzene and ethyl acetate (85:15), air-dried sprayed with50% H_2SO_4 and subsequently heated at. Two colours were matched with the standard samples of β-Sitosterol (Rf.0.52) and Stigmasterols was (Rf. 0.49) were observed in the plant samples. A few other solvent systems (hexane: acetone, 80: 20; benzene: ethyl acetate, 3:1) were also tried but benzene and ethyl acetate (85:15) gave an excellent result in the present investigations.

Preparative thin layer chromatography (PLC)

Each of the extract along with standard reference sterols was applied separately on thickly silica gel (0.3mm-0.4mm) G coated and activated glass plates. The plates were developed in an organic solvent mixture of benzene and ethyl acetate (85:15).

The developed plates were air dried and visualized under UV light. The two fluorescent spots(Rf. 0.49) and (Rf. 0.52) corresponding with β-Sitosterol Stigmasterol in roots, shoots and fruits of selected plant species and collected along with silica gel from unsprayed plates. Each of the mixtures was eluted with chloroform. The elute were dried in the vacuum crystallize separately with acetone and methanol.

Quantitative estimation

Quantitative estimation of β-sitosterol and stigmasterol in each of the test sample was carried out using the method of Das and Banerjee (1980). A stock solution of β-sitosterol and stigmasterol in chloroform (500 micro gm/ml) was separately prepared, from this 6 conc. (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6) were prepared and spotted on silica gel G coated on an activated glass plate. The plates were developed in the solvent system of benzene and ethyl acetate (85:15). Such developed chromatographs were air-dried and exposed to iodine vapours. Iodine spots were marked and heated to evaporate excess of iodine. The spots were scrapped along with silica gel and each was eluted with 5ml of chloroform in test tubes. Each of the tubes was centrifuged, supernatants were taken, solution evaporated to dryness and processed further. To each of the dried samples, 3ml of glacial acetic acid was added and shaken on a vertex mixture at room temperature for 1min and then immersed in crushed ice. To this frozen sample, 2ml freshly prepared chromogenic reagents(0.5ml of 0.5% anhydrous ferric chloride in glacial acetic acid and 100ml of conc. Sulphuric acid; Klyne, 1965) was added drop wise at 0ºC and mixed thoroughly. Each of the reaction mixtures was incubi ted at 40 ºC for 30 min. The optical density of each of the test sample was taken in a spectronic -20colorimeters set at 540nm against a blank (3ml glacial acetic acid and 2mlof chromogenic reagent). Five such replicates run for each of conc.to minimize the standard deviation and average optical density was plotted against their respected conc. To complete a regression curve, which follows bear law.

Each of the extracts was spotted along with β-sitosterol and stigmasterol on the silica gel G activated glass plate and developed in benzene and ethyl acetate (85:15). The two spots coincided with those of the authentic of β-sitosterol and stigmasterol were marked in all samples of selected plant species. Each of the elute was dried, taken up in 5ml of chloroform and r as described above, conc. of β-sitosterol and stigmasterol were calculated (mg/g d.w.) by comparing the

optical density of standard β-sitosterol and stigmasterol separately. Five such replicates were examined in each case and the mean values calculated.

Table. 2.Sterol content in Eclipta prostrata and Pendalium murex

Name of Sterol	Eclipta prostrata	Pendalium murex
	Fruits	Roots
β-Sitosterol	2.12mg/g d.w	1.04mg/g d.w
	Fruits	Roots
Stigmasterol	0.92mg/g d.w	0.69mg/g d.w
Total sterol content	3.04mg/g d.w	1.73mg/g d.w

Result and discussion:

Medicinal Aspect of Eclipta prostrata:

Bhringaraja powder one part, black sesame seeds half part and Aamalaka (Emblica officinale) half part was prescribed as an age sustaining tonic. It is used as a detoxifying antiseptic herb in animal for Liver enlargements Hyperacidity and Dysentery the juice of Bhringaraja is used to wash the wounds. The oil extract of leaves is used for hair growth and gives natural colour to grey hairs. Its seeds are used in sex debility and as a tonic. Its juice followed by milk retards old age and reduce old age problems.

Medicinal Aspect of Pendalium murex:

A simple decoction of Gokhru seeds mixed with honey or a paste of Gokhru with coconut water is prescribed for dysuria. Gokhru is recommended for painful micturition, suppression of urine. It's decoction in milk with Amla, Shatavari and Sesame seeds work as a sex tonic. A decoction of Ghokru and Shunthi is prescribed for rheumatism. Gokhru is an ingredient for Dashmularistha due to its diuretic property. Dashmularistha is a restorative tonic for women suffering from urinogenital diseases.

Flavonoids: Presence of Two Flavonoids Quercetin (Rf 0.82 bluish yellow) and Kaempferol (Rf 0.93 bright yellowish blue) was confirmed in samples 2 and 3 the presence of Quercetin and Kaempferol was confirmed.

Quercetin is maximum (1.10 mg/g d.w.) in the leaves of Eclipta prostrata and minimum (0.10mg/g d.w.) in the leaves of Pendalium murex. Similarly, Kaempferol is maximum (5.20 mg/g d.w) in the leaves of Eclipta prostrata and minimum (3.20 mg/g d.w.) in the fruits of Pendalium murex as shown in Table.1.

Sterols: β – sitosterol and stigmasterol were confirmed by Co- TLC.(Rf βsitosterol 0.52 and stigmasterol, 0.49)M.pt (Beta-sitosterol, 139-140 °C stigmasterol 132-133 °C) and superimposable IR spectra of isolated and the authentic samples of sterol. Maximum sterols content was observed in fruits of Eclipta prostrata (3.04mg /g d.w.) when compared with other plant parts of

Pendalium murex, where as it is minimum in the roots of Pendalium murex (1.73mg/g d.w.) as seen in Table. 2

β– Sitosterol contents is maximum (2.14 mg/g d.w.) in the fruits of Eclipta prostrata and minimum (1.04 mg/g d.w.) in the roots of Pendalium murex. Stigmasterol contents are maximum (0.92mg/g d.w.) in the fruits of Eclipta prostrata and minimum (0.69mg/g d.w.) in the roots of Pendalium murex as seen in Tasble.2

This work of Eclipta Prostrata was compared with phytochemical analysis obtained by Gas chromatography coupled with mass spectrometry in other species Eclipta alba. It is observed that eight possible bioactive compounds were found such as Tridecanol, 2-ethyl-2methyl, 1-Hepta triacotanol, c-sitosterol, oleic acid, eicosyl ester etc.,These phytochemical compounds contains anti-inflamatory, cancer preventive dermatitigenic, Hypocholestrolemic and anaemiagicinsectifuge. Similarly, Eclipta prostrata contains chemical constituent namely oleanone-type glycosides Eclaibasaponin I and Eclaibasaponin II which is used in manufacturing drugs for treatments of many diseases.(John wyson., M.Devithiran., Saravanan Periswamy., Devireddy Anand., Jan.,2016).

This work of Pendalium murex was matched with phytochemical analysis of Pendalium murex by M.Sermakkani and V.Thangapandian ,They carried out the analysis according to standard method(M.Sermakkani., V.Thangapandian.,2010). The analysis showed that the leaves and fruit extracts of Pendalium contains Flavonoids, alkaloids,glycosides, sterols,phenols,saponins,terpenoids glycosides etc. Flavonoids and Tannins are phenolic compounds they act as antioxidants and free radical scavengers(Polterait,1997),Plants derived natural products such as Flavonoids,terpenoids and steroid have received considerable attention in recent years due to their diverse pharmacological properties and can be used as a source of potential drugs.

This investigation indicates that sterol can be isolated from intact part of medicinal plant species growing at Kalanaur area of Rohtak district. The medicinal plant of this zone has sufficient amount of sterol content which can be a good source of Phytosterol.

Conclusion

The Result shows that Eclipta prostrata is rich in Flavonoid and Sterol contents as compared to Pendalium murex.

Sufficient amount of sterol shows that these weeds are a good source of Phytosterol, and are of good medicinal value and Nature-based formulations for Pharmaceutical Industries.

Farming of these plants can fetch business to Farmers and generate Revenue to the Nation.

Acknowledgement
I would like to thank my family Mr.Alok Kumar Roy for his inspiration, expert advice and esteemed support and Mrs.Vrushali Gaikwad and Dr.Kunal Khobragade for motivation and encouragement. Miss Samiksha Garse for her versatile approach and encouragement.
I am truly grateful and my exclusively special thanks to Dr. Minakshi Gurav my "Friend philosopher and guide "for her valuable guidance and support without her this would not be possible.

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