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A STUDY ON THE BIOACTIVE COMPOUNDS PRESENT IN LEAVES OF DICHROSTACHYS CINEREA

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ABSTRACT

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug because of the unmatched availability of chemical diversity. Due to an increasing demand for chemical diversity, seeking therapeutic drugs from natural products interest particularly in edible plants has grown throughout the world. Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds. The focus of this paper is on the analytical methodologies, which include the extraction, isolation and characterization of active components in leaves of Dichrostachys cinerea. The analysis of bioactive compounds present in the plant extracts involve the application of common phytochemical screening tests, physicochemical tests, chromatographic techniques such as HPLC and TLC were discussed.

Keywords: Dichrostachys cinerea, physicochemical, phytochemical, TLC, bioactive

INTRODUCTION

Plants have been used in traditional medicine for several thousands of years [1].The traditional medicinal plant Dichrostachys cinerea is a medium sized tree commonly distributed in the forest of Africa, Australia, India and parts of South East Asia[2]. Various studies on medicinal plant Dichrostachys cinerea showed that it possess various pharmacological activities namely antibacterial activity, acute toxicity, hepatoprotective property, antioxidant activity, anti- inflammatory, safety and toxicity, anti-mosquito activities [2,3]. These pharmacological activities are mainly due to the active components present in the plant. Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds [4]since medicinal herbs have been used in one form or another under indigenous systems of medicine. Dubeyetal. [5] Mentioned that the complete phytochemical investigations of medicinal plants of India should be carried out, because these secondary metabolites are responsible for medicinal activity of the plant. These secondary metabolites present can be derived from any part of the plant [6], but various parts of medicinal plant especially leaves, stem, flowers are attacked by bacterial stains, pathogens etc. The WHO encourages the use of traditional drugs because of its less side effects and most of the European countries were expanding towards Ayurvedic medicine[7].This lead the developing countries and industrialized societies to extraction and development of several new drugs from plants as well as from traditionally used folk medicine[8]. Since medicinal plants constitute the main source of new pharmaceuticals and health care products [9], quality control methods are of utmost importance for maintenance of quality of herbal medicines. The various parameters like total ash content, acid insoluble ash, water soluble ash and sulphated ash act as a valuable tool for maintaining the quality of herbal medicines[10]. It is a common practice in isolation of these bioactive compounds that a number of different separation techniques such as Thin-Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), should be used to obtain pure compounds [11]. So more work has to be done on the medicinal plant. Thus the proposed work was aimed to determine the various active components present in leaf extracts of Dichrostachys cinerea and techniques in determining the active components.

MATERIAL AND METHODS

Chemicals

Hexane, chloroform, Ethyl acetate, Acetone, Methanol

Physico-chemical Analysis

The samples were subjected to physicochemical analysis according to standard procedures as provided in literature [12, 13].

Determination of total Ash

A known quantity of dried samples of leaves of *Dichrostachys cinerea* was taken in previously weighed silica crucible and ignited carefully not exceeding dull red heat until the ash was free from carbon. The crucible was cooled and weighed. The percentage of ash with reference to the air- dried was calculated.

Determination of water soluble ash

A known weight of the ash (0.2g) was boiled with 25ml of distilled water. The insoluble matter was collected in a previously weighed sintered crucible, washed with hot water, dried to a constant weight and weighed. The percentage of water soluble ash with reference to air – dried sample was calculated.

GIAP Journals

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Determination of acid- insoluble ash

A known weight of the ash (about 0.1g) was boiled with 25ml of dil. HCl (2N). The insoluble matter was collected in a previously weighed sintered crucible washed with hot water, dried to constant weight and weighed. The percentage of acid insoluble ash with reference to air – dried sample was calculated.

Determination of residue on ignition

A known weight of the air dried samples (2g) was taken in a previously weighed silica crucible and carefully incinerated till the ash was strongly ignited, cooled and weighed. The percentage of ignited ash with reference to air – dried sample was calculated.

Determination of sulphated ash

Take 1g of substance in a crucible and weigh the contents accurately. Ignite gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1ml of sulphuric acid, heat gently until the white fumes are no longer evolved and ignite at 800^0 +. 25^0 until all black particles have disappeared. Conduct the ignition in a place protected from air currents. Allow the crucible to cool, add a few drops of sulphuric acid and heat. Ignite as before, allow to cool weigh. Repeat the operation until two successive weighing do not differ by more than 0.5mg.Table:1

Preliminary phytochemical screening Analysis

The different extracts were subjected to preliminary phytochemical screening techniques provided in literature [14-15].

Test for alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Test for Saponins

Froth test

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for proteins

Millon's test

Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Test for glycosides

Liebermann's test

Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Salkowski's test

Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

Test for steroids

Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish colouration indicated the presence of steroids.

Test for flavonoids

Shinoda test

Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.

GIAP Journals
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Alkaline reagent test

Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for carbohydrates

Molisch's test

Crude extract was mixed with 2ml of Molisch's reagent and the mixture was shaken properly. After that, 2ml of concentrated H_2SO_4 was poured carefully along the side of the test tube. Appearance of a violet ring at the inter phase indicated the presence of carbohydrate

Detection of triterpenes

Salkowski's Test

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Concentrated Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Detection of phytosterols

Libermann Burchard's test

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of diterpenes

Copper acetate Test

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Detection of tannins

Gelatin Test

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of coumarins 10% NaOH (1ml) was added to 1 ml of the plant extracts formation of yellow color indicated presence of coumarins. Table :2

Plant material

The leaves of *Dichrostachys cinerea* were collected from the regions of Kalakadu, Trichendur, Tirunelvelli Districts, Tamil Nadu and the specimens voucher were deposited to Dr. Jeeva, Department of Botany, Scott Christian college Autonomous Nagercoil for identification(voucher no. 3268). The collected leaves of *Dichrostachys cinerea* were dried shade in a dark room and have powdered in an electric homogenizer. The powdered samples were extracted with different solvents like Hexane, chloroform, Ethyl acetate, Acetone, Methanol in soxhlet apparatus for 24 hours.

TLC and HPLC technique

The chloroform extract of *Dichrostachys cinerea* leaves was chosen for the study. The ratio of 9:1 (chloroform: methanol) is selected for detecting the spots and colour of spots found using UV Light. The HPLC technique performed on chloroform extract showed three components at peak area of 81.53, 16.26, 2.21. The results were recorded in tabular column. TABLE: 3

RESULT AND DISCUSSION

A large number of plants produce secondary metabolites such as alkaloids, flavonoids, terpenes, steroids that are used in pharmaceuticals, cosmetics and pesticide industries [16]. Flavonoids have been reported to possess many useful properties, including anti- inflammatory, oestrogenic, enzyme inhibition, antimicrobial, anti-allergic, antioxidant, vascular and cytotoxic antitumor activity [17, 18]. Traditionally Saponins have been extensively used as detergents, pesticides and molluscides, in addition to their industrial applications as foaming and surface active agents and also have beneficial health effects [19]. The present study has done phytochemical analysis on Dichrostachys cinerea to find the secondary metabolites on different extracts namely Hexane, Chloroform, Ethyl Acetate, Acetone, Methanol. A large number of secondary metabolites were mostly found in methanol extract. Saponins were found in chloroform extract. Likewise flavonoids were found in acetone extract. The results of the present study have revealed the

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presence of these secondary metabolites in the leaves of Dichrostachys cinerea. By isolating and identifying these bioactive compounds new drugs can be formulated to treat various diseases and disorders [16]. Accounting to WHO, quality control is the process involving the physicochemical evaluation of crude drug covering the aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion. Four types of ash values used in routine pharmaceutical analysis are total ash content, acid insoluble ash, water soluble ash and sulphated ash [10].In our physicochemical analysis the ash values were found as follows 25.85%, 13.3%, 21.45%, 38.55%, 12.15%. This was done to maintain the quality of crude drug which can be prepared from the traditional plant. In TLC ratio of 9:1 (chloroform: methanol) is selected for detecting the spots and colour of spots is found using UV light. In short wave length three spots were obtained and in long wavelength four spots were obtained with multi colours. Thus TLC has been used for identifying the components and conforming their purity. The chloroform extract filtrate is then concentrated and injected into HPLC for separation at a volume of 10. Three components were identified. Thus HPLC technique is used for maintaining the quality of medicinal plant.

CONCLUSION AND FUTURE SCOPE OF WORK

Today many countries in the world are approaching to Ayurvedic medicine because of its less side effects. Thus it is essential to do pharmacological investigation on the medicinal plant taken for our study. The physicochemical analysis done on Dichrostachys cinerea leaves showed that new drugs can be prepared from the medicinal plant of high quality. From the phytochemical investigation done on the medicinal plant, it is found that they have marked role on the industrial and health effects. Since bioactive compounds occurring in plant material appear as multi coloured spots, their separation and determination still creates problems. So most of them have to be purified by the combination of several chromatographic techniques and various other purification methods to isolate bioactive components.

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Table: 1 Physicochemical Analysis of Ash values of Dichrostachys cinerea leaves

Sl. No	Parameters	Percentage of ash values
1	Total ash	25.85
2	Water soluble ash	13.3
3	Acid insoluble ash	21.45
4	Sulphated ash	38.55
5	Residue on ignition	12.15

Table : 2 Phytochemical evaluation of *Dichrostachys cinerea* leaves

Sl. No	Name of extract	Alkaloid	Sapoinin	Protein	Glycoside	Steroid	Flavonoid	Carbohydrate	Triterpenes	Phytosterols	Diterpenes	Tannis	Coumarins
1	Hexane	-	-	-	-	-	-	-	-	+	-	-	-
2	Chloroform	+	-	-	-	-	-	-	-	-	-	-	-
3	Ethyl Acetate	-	-	-	-	-	-	-	-	-	+	-	+
4	Acetone	-	-	+	-	-	+	-	-	-	-	-	-
5	Methanol	-	-	-	+	-	-	+	+	-	-	+	+

Where, + indicates the presence of secondary metabolites, - indicates the absence of secondary metabolites

GIAP Journals

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Table: 3 Rf values and colour of chloroform extract of *Dichrostachys cinerea* leaves viewed in UV light

Sl. No	Wave length	Chloroform extract of *Dichrostachys cinerea* leaves	
		R_f values	Colour
1	Short wave length	0.2 0.8 0.9	Light green green fluorescence green
2	Long wave length	0.2 0.6 0.8 0.9	brown pink brown light brown

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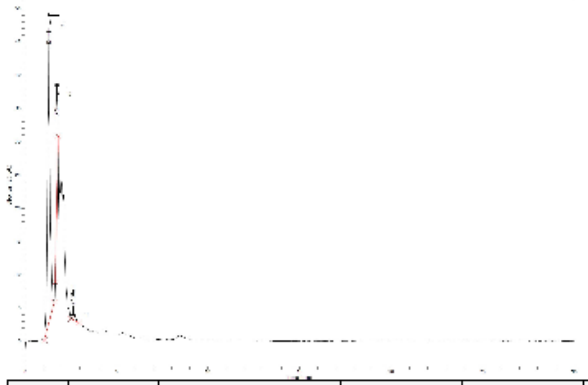
Chromera Version	3.3.0.5361
Instrument Name	Flexar
Report Date/Time	4/23/2013 1:13:05 PM
Sample Description	
Sample Name	Anitha 1
Processing Method	Anitha
Injection Volume	10



Peak #	Time	Component Name	Area	Height	Area	Final Amount
1	1.321	a1	395,357.6	46,008.9	81.53	
2	1.728	a2	78,823.8	18,334.3	16.26	
3	2.636	a3	10,727.3	852.6	2.21	
Total			484,908.7		100.00	

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Green Chemistry & Technology Letters
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REFERENCES

1. K. Shubashini. Sripathi and Uma Sankari, Ethnobotanical Documentation of a few Medicinal plants in the Agasthiayamalai Region of Tirunelveli District India Ethnobotanical leaflets, 14, 2014, 173-181.
2. Babu et.al., Evaluation of actue toxicity and hepatoprotective activity of the methanolic extract of Dichrostachys cinerea (Wight and Arn.) leaves, Pharmacognosy Research, 3(1), 2011, 40-43.
3. Hurinanthan, Immune modulatory effect of Dichrostachys cinerea ,Carpobrotus dimidiatus, Capparisto mentosa and Leonotis leonurus, 2009, 116-121.
4. Akindele and Adeyemi , Anti- inflammatory activity of the aqueous leaf extract of Byrsocarpuscoccineus, Fitoterapia, 78, 2007, 25-28.
5. N. K. Dubey, Rajesh Kumar and Pramila Tripathi, Global promotion of herbal medicine: India's opportunity, Current science, 86(1), 2004, 37-41.
6. Gordon M. Cragg and David J. Newman, Natural product drug discovery in the next millennium, Journal of pharmaceutical Biology 39(1), 2001, 8-17.
7. Savithramma et.al., Screening of medicinal plants for secondary metabolites, Middle – East Journal of Scientific Research,(8), (3), (2011), 579-584.
8. Sapna Shrikumar, T. K. Rav, Approaches towards development and promotion of herbal drugs, pharmacognosy review 1(1), 2007, 180-184.
9. Savithramma et.al., Phytochemical screening of traditional medicinal plants. Journal of Pharmacy Research 4(10), 2011, 3414-3416.
10. Mammenet.al., Variations in values of proximate analysis in Aerva lanata Juss Ex schultes, Hedyotis corymbosa (L.) LAM and Leptadenia Reticulata (Retz.)W.&A, International Journal of Pharma & Bio Science 1(4), 2010, 629-636.
11. Michel Martin and Georges Guiochon, Effects of high pressure in liquid chromatography. Journal of Chromatography 2005(1090), (1-2), 2005,16-38.
12. A. Jeena Pearl, Phytochemical studies in the leaves of Ecbolcium linneanum- The medicinal plant, International Journal of Research in Engineering and Bioscience, 1(1), 2013, 111-117.
13. Ankur Choudhary,Test for sulphated ash and Total ash. (ONLINE) Available at: htpp:\\www.Pharmaguideline.com/2011/06/test- for- sulphated ash and – total-ash.html l(Accessed on 19, Jan 2015), 2011
14. Prashant et.al.,. Phytochemical screening and Extraction: A Review, Internationale Pharmaceutica Sciencia 1(1), 2011, 98-105.
15. Vijajlakshmi and Ravindhran., Preliminary comparative and phytochemical screening of root extracts of Diospyrus ferrea (Wild.)Bakh and Aerva lanata (L) Juss. Ex Schultes. Asian Journal of Plant Science and Research 2(5), 2012, 581-587.
16. Yamunadevi Mariswamy, Wesely Edward Gnaraj, M Johnson, Chromatog raphic finger print analysis of steroids in Aerva lanata.L by HPTLC technique , Asian Journal of Tropical Biomedicine 1(6), 2011, 428-433.
17. Havesteen, Flavonoids a class of natural products for antimicrobial agents. Eur J clin Microbiol Infect Dis (9), 1990, 455-461.
18. Harbone and Williams,. Advances in Flavonoids research since 1992. Phytochemistry (55), 2000, 481-504.
19. Shi et.al ., Saponins from edible legumes: Chemistry, processing and health benefits, J Med Food, 7(1), 2004, 67-78.