IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Phytochemical Screening And Extraction Of Products From Grass

[1] Sandeep Thakur, [2] Vijay Sangore, [3]Nikhil Ghare, [4]Vijay Diware, [5]Sanjay Bhagat

[1,2,3]Assistant Professor, [4]Associate Professor, SSBT's College of Engineering & Technology, Bambhori, Jalgaon, Maharashtra (India), [5]Associate Professor, PVP Institute of Technology, Sangli, Maharashtra (India).

ABSTRACT:

Phytochemicals are chemical compounds that occur naturally in plants. Plant based medicines are important for curing diseases. The medicinal value of plant lies in the bioactive phytochemical constituents of the plant. Phytochemical screening is one of the technique to identify new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolics, steroids, tannins, saponins etc. present in the plant extracts. These compounds can be derived from any part of the plants like bark, leaves, flowers, seeds, etc. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of new bioactive compound/s for treating the specific disease.

The study of phytochemical screening is a step towards identifying the various important compounds which could be used as the base of modern drugs for curing various diseases. Different solvents are used under a time and temperature condition for extraction. Gas Chromatographic Analysis confirmed the presence of important phytochemicals in the Grass sample considered for the study.

Key words—Phytochemical screening, Extraction, Grass

INTRODUCTION:

Phytochemicals are compounds that are made by plants and have many health benefits, although they are not necessary to sustain life like other vitamins and minerals. Plant based foods, including fruits, vegetables, nuts, seeds, legumes, and grains may contain hundreds of different phytochemicals. Phytochemicals are chemical compounds that occur naturally in plants. Phytochemicals, as plant components with discrete bio-activities towards animal biochemistry and metabolism are being widely examined for their ability to provide health benefits. It is important to establish the scientific rationale to defend their use in foods, as potential nutritionally active ingredients. [2]

Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds. Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. Levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions. Phytochemicals are also available in supplementary forms, but evidence is lacking that they provide the same health benefits as dietary phytochemicals. [5,6]

There are about 4000 and more than these phytochemicals avaiolable, some important amongst them are alkaloids, glycosides, flavonoids, phenolics, saponins, tannins, terpenes etc. [13]

EXTRACTION OF PHYTOCHEMICALS:

Extraction: Extraction is a separation process consisting in the separation of a substance from a matrix. For phytochemical extraction from grass, no single method is sufficient to study the bioactive of phytochemicals from a given plant. An appropriate assay is required to first screen for the presence of the source material, to purify and subsequently identify the compound therein. Extraction from the plant is empirical exercises in different solvents are utilized under a variety of condition such as time and temperature of extraction. Once extracted from the plant the bioactive components then has to be separated from the co extractives. Further purification steps may involve simple crystallization of the compound from the crude extract, further solvent partition of the co extractive or chromatographic methods in order to fractionate the compounds based on their acidity polarity, or molecular size. Final purification, to provide compounds of suitable purity for such structural analysis, may be accomplished by appropriate techniques such as recrystallization, sublimation, or distillation.

Solvent Extraction: Various solvents have been used to extract different phytoconstituents. The plant parts are dried immediately either in an artificial environment at low temperature (45-50°C) or dried preferably in shade so as to bring down the initial large moisture content to enable its prolonged storage life and . The dried berries are pulverized by mechanical grinders or mixer and the oil is removed by solvent extraction. The defatted material is then extracted in a soxhlet apparatus or by soaking in water or alcohol. The resulting alcoholic extract is filtered, concentrated in by evaporation, treated with HCl and refluxed for at least six hours. This can then be concentrated and used to determine the presence of phytoconstituents. Generally, the saponins do have high molecular weight and hence their isolation in the purest form poses some practical difficulties.

Exhaustive Extraction (EE) is usually carried out with different solvents of increasing polarity in order to extract as much as possible the most active components with highest biological activity.

The Soxhlet apparatus is used for the extraction of phytochemicals Typically, a Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days.

During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

After extraction the solvent is removed, typically by means of a rotary evaporator or simple distillation, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

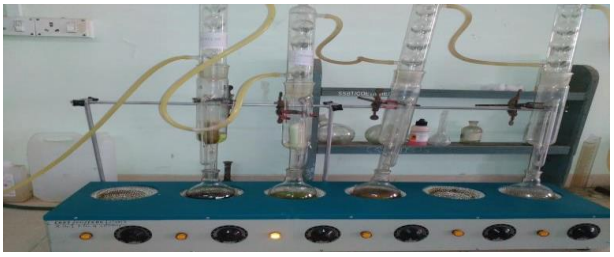

Fig. 1: Soxhlet Apparatus

Fig. 2 : Distillation Assembly

SCREENING OF PHYTOCHEMICALS:

Screening is the process of separation and isolation of active principle from plant sources. Screening is helpful-

- To get lead for Discovery of new therapeutic agents.
- To find new sources for economic material.
- To help expand chemotaxonomy.
- To produce “semisynthetic” derivatives.

For this purpose, following 3 essential steps are prescribed- Selection of plant, Phytochemicals screening. Phytopharmacological evaluation. [3, 7, 9, 10]. A method for use in phytochemical screening should be simple, rapid, designed for minimum of equipment, reasonably selective for the class of compounds under study, quantitative in so far as having a knowledge of the lower limit of detection is concerned, and if possible should give additional information as to the presence or absence of specific members of the group being evaluated.

QUALITATIVE PHYTOCHEMICALS ANALYSIS FOR DIFFERENT EXTRACTS OF SOLVENTS [4, 11, 12]:

- **Alkaloids (Wagner’s reagent)**

  A fraction of extract was treated with 3-5drops of Wagner’s reagent [1.27g of iodine and 2g of potassium iodide in100ml of water] and observed for the formation of reddish brown precipitate (or colouration).

- **Carbohydrates (Molisch's test)**

  Few drops of Molisch's reagent were added to 2ml of the extracts. This was followed by addition of 2ml of conc. H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for two-three minutes. Formation of a red or dull violet colour at the interphase of the two layers was a positive test.

- **Cardiac glycosides (Keller Kelliani's test)**

  5ml of each extract was treated with 2ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayed with 1ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form

- **Flavonoids (Alkaline reagent test)**

  2ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

- **Phenols (Ferric chloride test)**

  A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour

- **Proteins**

  Biuret's test: To 1ml of extracts of Durva churna taken in separate test tubes, 5-8 drops of 10% NaOH solution, followed by 1- 2 drops of 3% $CuSO_4$ was added. Formation of violet colored solution indicates the presence of proteins

- **Saponins** (Foam test)

  To 2mls of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

- **Tannins** (Braymer's test)

  2mls of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

- **Terpenoids** (Salkowki's test)

  1ml of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.[1,6]

The different extraction solvents used for the study are Benzene, Hexane, Ethanol and Methanol and for Identification of the components present, gas chromatography technique has been used.

RESULTS & DISCUSSION:

Following identification and observations are marked during Phytochemical Screening and Extraction of Products from Grass with Benzene, Hexane, Ethanol and Methanol.

1. Raw Sample: Benzene Extract

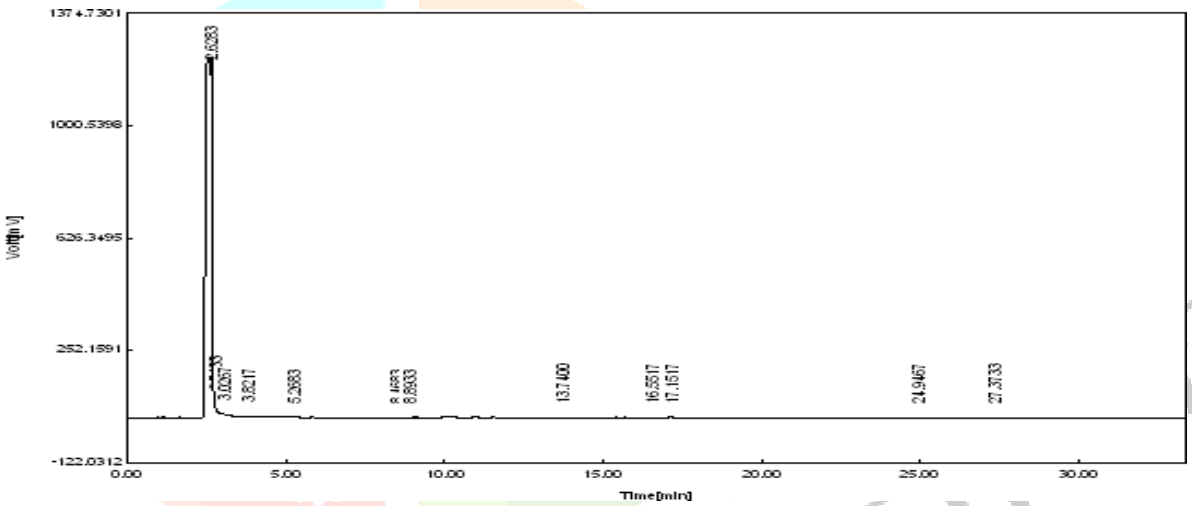

Chart 1: Chromatographic Analysis of the extract using Benzene as solvent

Result			
Index	RT[min]	Area[mV*s]	Area%
1	2.5017	11146.2558	69.8616
2	2.6283	4759.1684	29.8291
3	2.7433	28.9932	0.1817
4	3.0267	6.4979	0.0407
5	3.8217	4.7937	0.0300
6	5.2517	0.1059	0.0007
7	5.2683	0.1037	0.0007
8	8.4683	0.2220	0.0014
9	8.8933	0.1080	0.0007
10	13.7400	0.1190	0.0007
11	16.5517	0.1547	0.0010
12	17.1517	7.9885	0.0501
13	24.9467	0.1292	0.0008
14	27.3733	0.1278	0.0008

Table 1: Chromatographic Result of the sample raw extract with Benzene as a solvent.

From Table 1 , Extract chemical components are compared on the basis of retention time of chromatographic peaks and tabulated in Table 2

Sr no	Retention Time(min)	Area(mV*S)	Area %	Identified components
1	16.5517	0.1547	0.0010	Tricosane
2	17.1517	7.9885	0.0501	Trans-Phytol
3	24.9467	0.1292	0.0008	Butyladehyde,4-benzyloxy-4-[2,2,-dimethyl-4-dioxolanyl]

Table 2: Identified Component from raw extract with Benzene as a solvent

2. Raw Sample: Hexane Extract

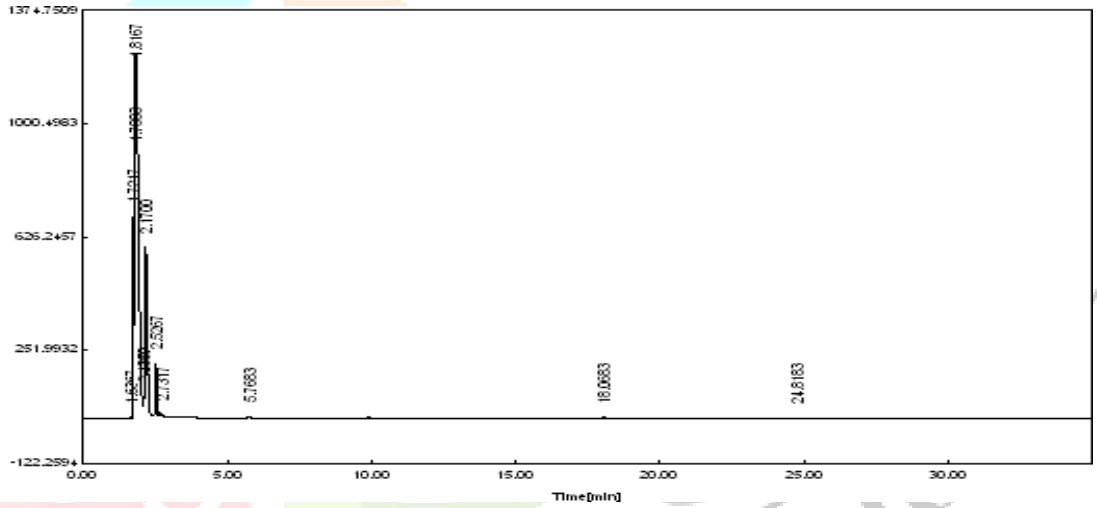

Chart 2: Chromatographic Analysis of the extract using Hexane as solvent

Result

Index	RT[min]	Area[mV*s]	Area %
1	1.6367	2.3580	0.0140
2	1.7217	1807.3600	10.7531
3	1.7883	1677.9319	9.9830
4	1.8167	9584.0835	57.0215
5	2.1350	215.7907	1.2839
6	2.1700	2707.7307	16.1099
7	2.4300	28.1084	0.1672
8	2.5267	654.9957	3.8970
9	2.6767	56.0409	0.3334
10	2.7317	22.5258	0.1340
11	5.7683	10.1392	0.0603
12	18.0683	25.0643	0.1491
13	24.8183	15.7001	0.0934

Table 3: Chromatographic Result of the sample raw extract with Hexane as a solvent.

From Table 3, Extract chemical components are compared on the basis of retention time of chromatographic peaks and tabulated in Table 4

Sr no	Retention Time(min)	Area(mV*S)	Area %	Identified components
1	18.0683	25.0643	0.1491	Phosphine Oxide,Bis(Pentamethylphenyl)
2	24.8183	15.7001	0.0934	Buteladehyde,4-benzyloxy-4-[2,2-dimethyl-4-dioxolanyl]

Table 4: Identified Component from raw extract with Benzene as a solvent

3. Raw Sample: Ethanol Extract

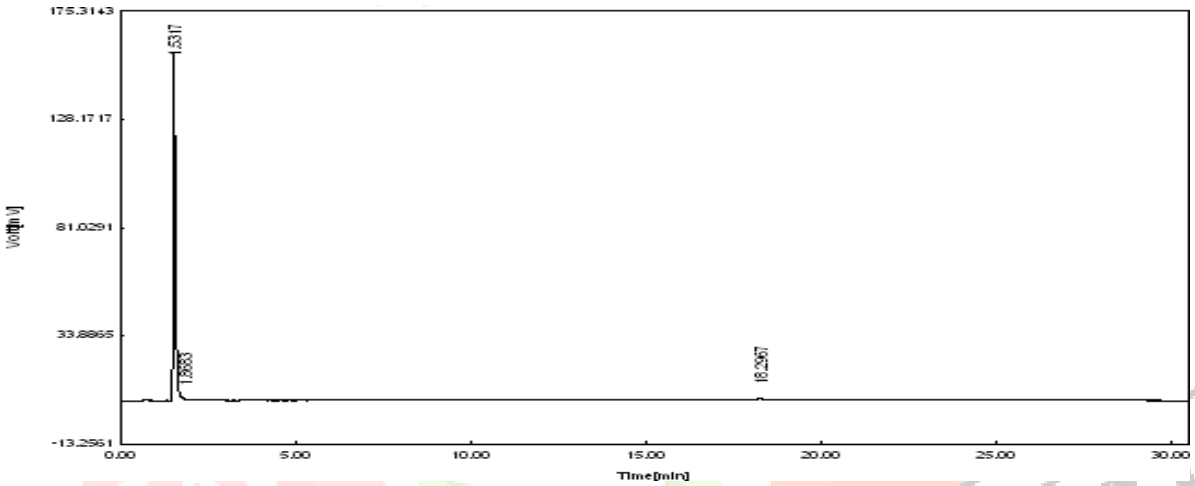

Chart 3: Chromatographic Analysis of the extract using Ethanol as solvent

Result

Index	RT[min]	Area[mV*s]	Area%
1	1.5317	644.0821	93.4613
2	1.8683	1.0796	0.1567
3	18.2967	43.9814	6.3820

Table 5: Chromatographic Result of the sample raw extract with Ethanol as a solvent.

From Table 5, Extract chemical components are compared on the basis of retention time of chromatographic peaks and tabulated in Table 6

Sr no	Retention Time(min)	Area(mV*S)	Area %	Identified components
1	18.2967	43.9814	6.3820	Dibutyl pthalate

Table 6: Identified Component from raw extract with Ethanol as a solvent

4. Raw Sample: Methanolic Extract

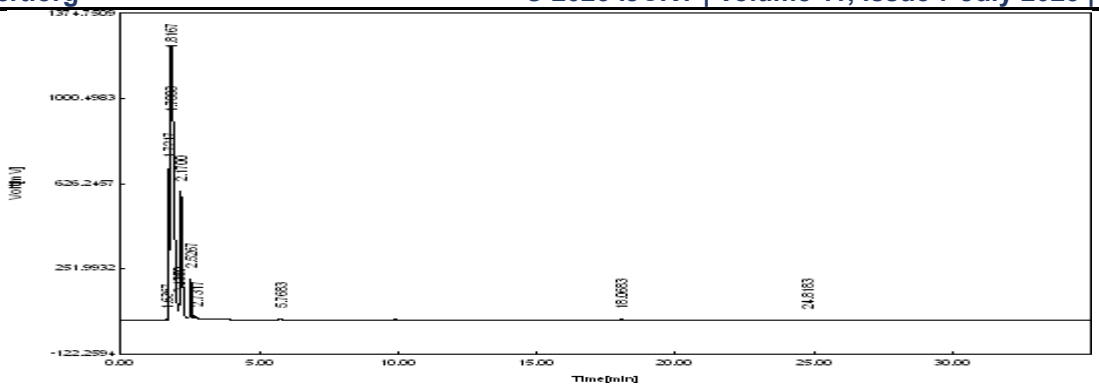

Chart 4: Chromatographic Analysis of the extract using Methanol as solvent

Result

Index	RT[min]	Area[mV*s]	Area%
1	0.8083	2.8689	0.1121
2	1.2833	1075.9732	42.0576
3	1.3817	1468.0406	57.3827
4	1.8950	7.7686	0.3037
5	1.9517	10.5913	0.4140
6	2.1117	2.2316	0.0872
7	18.4517	9.0757	0.3547
8	21.2350	1.5695	0.0613
9	28.7517	1.5044	0.0588

Table 7: Chromatographic Result of the sample raw extract with Methanol as a solvent.

From Table 7, Extract chemical components are compared on the basis of retention time of chromatographic peaks and tabulated in Table 8

Sr no	Retention Time(min)	Area(mV*S)	Area %	Identified components
1	18.4517	9.0757	0.3547	Phosphine Oxide,Bis(Pentamethylphenyl)
2	21.2350	1.5695	0.0613	1-Adamantanecarboxylicacid,2-Isopropoxyphenyl ester
3	28.7517	1.5044	0.0588	Stgmast-5-3Ol, Oleat

Table 6: Identified Component from raw extract with Ethanol as a solvent

Conclusion

Phytochemicals are successfully screened and extracted and identified, the key future of the available phytochemicals in cynodon dactylon is that it is derived almost entirely from all parts of plants such as fruit ,leaves , roots, stems etc. The presence of various phytochemicals such as flavonoids, terpenoids, saponin, steroid and cardiac glycosides in the different plants such as cynodon dactylon confirms that this genus is a potent source for modern drugs. As the species is weedy grass and cultivation is not essentiality, the species may easily be explored for human benefits from natural habitat.

In the qualitative test of ethanolic extract alkaloids, tannins ,iodine, phenols was succesfully screened out in addition with dibutyl pthalete was identified by GC. From all present phytochemical findings suggest that, the plant widely available could be a prominent source of medicinally important natural compounds.

In the qualitative testing of analysis of benzene extract phenols and saponins was easily screened out. And by GC analysis Butyladehyde,4-benzyloxy-4-[2,2,-dimethyl-4- dioxolanyl], Trans-phytol, and Tricosane these contents were present. In the qualitative analysis of Methanol extract Alkaloids ,Glycosides, Carbohydrates, Flavonoids , Saponnins , tannins and terpenes were present And by GC analysis Phosphine Oxide,Bis(Pentamethylphenyl), 1-Adamantanecarboxylicacid,2-Isopropoxyphenyl ester, Stigmast-5-En-3-Ol,Oleat were present. Similarly in Hexane extract ,Alkaloids, glycosides, carbohydrates and terpenoids were present and by GC-Analysis Butyladehyde,4-benzyloxy- 4-[2,2,-dimethyl-4-dioxolanyl], Phosphine Oxide,Bis(Pentamethylphenyl) were present.

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