EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 2
297-302
Year: 2017

PHYTOCHEMICAL PROFILING OF ETHANOLIC LEAVES EXTRACT OF *BRASSICA OLERACEA CAPITATA* DC.

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Article Received on 16/12/2016 **Article Revised on 06/01/2017** **Article Accepted on 26/01/2017**

ABSTRACT
Ethanol extracts leaves extract of *Brassica oleracea* capitata is a traditional medicinal plant and the leaves have tremendous medicinal values. In the present study ethanolic leaf extract of *Brassica oleracea* was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS) and the compound structures were identified with help of National Institute of Standards and Technology (NIST) library. GC-MS analysis of test plant revealed the presence of 20 bioactive compounds. Among them 2-Pentadecanone, 6,10,14-trimethyl-, -Hexadecanoic acid and 2-Hexadecen-1-ol, 3,7,11,15-Tetrame are important bioactive compounds which act as essential drugs for dangerous diseases and disorders and other compounds are used in antimicrobial, anti-inflammatory, cardioproductive, antioxidant, cytotoxicity and anticancer activities.

KEYWORDS: *Brassica oleracea*, GC-MS, Bioactive compounds.

INTRODUCTION
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, many of them based on their use in traditional medicine. Various medicinal plants have been used for daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible has been traced to the occurrence of natural products with medicinal properties. In fact plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry (Boominathan and Ramamurthy, 2009).

There has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom. Plants are the basic source of knowledge in modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. The worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care.

Broccoli is a form of cabbage, the *Brassica oleracea* capitata DC., or *Brassica oleracea* conica (H), of the mustard (Brassicaceae) family. It is a fast-growing, upright, branched, annual plant, 60-90 cm tall that is prized for its top crowns of tender, edible, green flower buds. Its thick, green stalks are edible too. It is native to Italy.

Broccoli and cauliflower are two derivatives of cabbage, both selected for their edible, immature flower heads. Broccoli is grown for the clustered green (or purple) flower buds that are picked before they open and eaten raw or cooked. The cauliflower head is a cluster of aborted, malformed flower buds that stopped developing in the bud stage. Cauliflowers come in white, lime green and purple varieties.

Broccoli has two different distinct forms. One is "sprouting broccoli," which makes a somewhat branching cluster of green flower buds atop a thick, green flower stalk, and smaller clusters that arise like "sprouts" from the stems. This form, called "calabrese" in Britain, is the most commonly grown form in the United States. The other type of broccoli makes a dense, white "curd" like that of cauliflower and is called "heading broccoli" or "cauliflower broccoli." This latter

form is usually grouped with cauliflower, leaving the term "broccoli" restricted to sprouting varieties.

Like the other close relatives of cabbage, broccoli is native to the Mediterranean area and Asia Minor. It has been popular in Italy since the days of the Roman Empire. However, records indicate this vegetable was unknown in England until a relatively recent few hundred years ago. It has become popular in the United States only during this century.

It thrives in moderate to cool climates and is propagated by seeds, either sown directly in the field or in plant beds to produce transplants. Broccoli grows to about 0.75 m high, and reaches harvest in 60 to 150 days, depending upon the variety and the weather. It is in flower from May to August, and the seeds ripen from July to September. The flowers are hermaphrodite (have both male and female organs) and are pollinated by bees. The plant can grow in semi-shade (light woodland) or no shade. It requires moist soil. The plant can tolerate maritime exposure.

Broccoli is available year-round but is a cool-weather vegetable that is best between January and March. Spring broccoli should be harvested in the early morning, because it wilts very rapidly in the sun. The broccoli head should be cut before the flower buds open. If the buds begin to open and the yellow flower petals begin to show, the head is over-mature and unfit for market. Cut the heads with a length of 23 to 25 cm from the base of the stem to the top of the head. The central heads vary from 6 to 12 cm in diameter. Light frosts do not hurt broccoli appreciably; therefore, harvest in the fall generally continues until the first freeze.

Medicinal plants are the source of many potent and powerful drugs. The plant derived drugs are healthier and safer alternate to the synthetic drugs (Dineshkumar and Rajakumar, 2015). Different parts of medicinal plants like root, stem, flower, fruit, seed etc. are used to obtain pharmacologically active constituents. Medicinal activities of plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, glycosides, tannins, terpenoids and essential amino acids present in these plants. These active principles are isolated for direct use as drugs, lead compounds and or pharmacological agents (Kumaradevan et al., 2015). Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries (Smolinske and Susan, 1992). Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material can be useful for proper standardization of herbals and its formulations. Also the WHO has emphasized the need to ensure the quality of medicinal plants products using modern controlled technique and applying suitable standards (Sharma et al., 2010). Nowadays there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation identification and structure determination of Phytochemicals. In GC-MS used to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc., Keeping this in view, the present study has been undertaken to investigate the identify the phytoconstituents present in ethanolic leaf extracts of *Brassica oleracea* using GC-MS analysis.

MATERIALS AND METHODS

Plant material and oil distillation

The medicinal plant of *Brassica oleracea* capitata DC leaves were collected from Chennai, Tamil Nadu, South India. The leaves were identified with the help of flora of presidency, Tamil Nadu and Karnatic flora (Gamble 1967; Matthew 1983) and standard references (Krtikar and Basu 1935).

Preparation of Extract

The dried and powdered leaves of *Brassica oleracea* (500 g) were extracted using soxhlet extractor by evaporating with 75% ethanol. The soxhlet extraction was carried out for 3 days and the extract was collected. The excess ethanol was evaporated by using vacuum evaporator. The sample is evaporated to dryness under boiling water bath at 55°C.

Phytochemical Analysis

The preliminary phytochemical evaluation of leaves was carried on extract prepared by successive extraction method in Soxhlet. The previously dried powdered (50 gm) were extracted in a Soxhlet apparatus with ethanol successively. The resultant extracts were evaporated to dryness under vacuum. These extract were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc. Chemical tests were identifying the phytochemicals as described by Sofowara (1993), Trease and Evans (1983), and Harborne (1973). Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Gas Chromatography-Mass spectrometry (GC-MS) analysis

The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µmdf capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised upto 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow

rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Identification of Compounds

The identity of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. National Institute of Standards and Technology library sources were also used for matching the identified components from the plant material.

RESULTS AND DISCUSSION

The result of phytochemical screening of the alcoholic extracts of *Brassica oleracea* revealed that the presence of alkaloids, flavanoids, phytosterols, tannins and phenols (Table 1). The plant extract of *Brassica oleracea* used for the present work was choosing on the basis of their medicinal values. The natural plant parts are having a wide range of medicinal properties like antimicrobial, diuretic, emollient, febrifuge, narcotic, purgative and sedative. Previous study in the naturally the ethanolic extracts of *Brassica oleracea* were subjected for phytochemical analysis. Phytochemical screening of the crude extract revealed that the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannin, flavonoids and steriods, but reducing sugars, carbonyl (aldehyde) and Phlobatanin show negative results (Makinde *et al*., 2007).

This plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized by several workers (Sofowara, 1993). In the present study, it was clearly understood that the alcohol extracted maximum amount of the different type of metabolites present in the *Brassica oleracea*. Boominathan and Ramamurthy (2009) reported that the phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins.

GC-MS analysis of ethanolic leaves extract of *Brassica oleracea* clearly showed the presence of twenty compounds with their molecular weight (MW), molecular structure and biological activities are presented in Table-2. The GC-MS chromatogram of the twenty peaks of the compounds detected was shown in Figure 1 and the components corresponding to the peaks were determined as follows: Diethyl Phthalate, Pentadecanal-, Oxirane, Tetradecyl-, (Z)6-Pentadecen-1-Ol, Tetradecanal, Decanoic Acid, 2,6,10-Trimethyl,14-Ethylene-14-Pe, 2-Pentadecanone, 6,10,14-Trimethyl-, Isoborneol, Cis-9-Hexadecenal, -Hexadecanoic Acid, 9-Octadecen-1-Ol, (Z)-, Z,Z-2,13-Octadecadien-1-Ol, Ethyl Pentadecanoate, Cyclohexane, 1-Ethenyl-1-Methyl-2,4-Bis(1-Me-, 1-Hexadecanol, 2-Hexadecen-1-Ol, 3,7,11,15-Tetrame, ,12-Octadecadienoic Acid (Z,Z)-, Tridecanedial, Linolenic Acid, 2-Hydroxy-1-(Hydroxymethyl)E, etc.,

The differences between the compounds that we have found in the roots, stems and leaves of *Aristolochia clematitis* were studied by GC-FID. This study was performed on the alcoholic extracts of the three parts of the plant. From this study we have concluded that the compounds found in the root and steam are very similar. The aristolochic acid derivatives are present in both extracts, but in the leaves these derivatives are in very low concentration (Podea *et al*., 2001). Ramamurthy et al. (2014) reported that the roots and leaves of *H. indicum* were studied by GC-MS. This study was performed on the alcoholic extracts of the two parts of the plant. From this study we have concluded that the compounds found in the root and leaves are very dissimilar. The organic acid derivatives are present in both extracts, but in the leaves these derivatives are in very high concentration.

Plants are integral part of human civilization. Medicinal plants are also been relied upon by over 80% of the world population for their basic health care needs. Drugs based on the Plants are of prime importance for several remedies in traditional and conventional medicine throughout the world and serves as a substitute for drug supply in modern medicine (Dineshkumar and Rajakumar, 2016). Medicinal plants with therapeutic properties are used for the treatment of many infectious diseases of humans as they contain many bioactive phytochemical constituents which are of curative effects. By consuming medicinal plants, can boost the immune system and increase antioxidant activity in humans. The high level of use as a medicinal plant due to easily available, cheap and relatively no side effects (Chandra et al., 2004).

1-hexodeconol is otherwise called as palmityl alcohol 1-hexadecanol is a fatty alcohol which are used in the cosmetic industry as an opacifier in shampoos or as an emulsifier or thickening agent in the manufacture of skin creams and lotions (pharmaceutical preparations) (Smolinske and Susan, 1992). It is one of the active ingredients in some "liquid pool covers". Dioctyl terephthalate (bis 2-ethylhexyl) benzene-1, 4-dicarboxylate is an organic compound is a general purpose plasticizer that is considered safer than *ortho*-phthalate plasticizers due to its excellent toxicological

profile. The terephthalates exhibit none of the peroxisome proliferation of liver enzymes that some *ortho*-phthalates have shown in several studies. It has uses in applications like extrusion, calendering, injection molding, rotational molding, dip molding, slush molding and coating.

Erucamide or 13 Docosenamide is an unsaturated long chain carboxylic acid amide is used as a slip agent, anti-fogging or lubricant for plastic films (polyolefin) which can be used in food packing material. It is used as a dispersant in printing and dying. It is used in paper and textile industry for water-proof as well as corrosion inhibitor in oil wells. It is used for the synthesis of organic chemicals and surfactants used in detergent, ore floating agent, fabric softener, anti-static agent, germicide, insecticide, emulsifier, anti-caking agent, lubricants and water treatment agent.

The analytical methods used GC/MS is suitable for medicinal herbs organic compounds determination. The sample preparation method is rapid and precise. There is a difference between the compounds extracted from herb by infusion and tincture but the important thing is that the organic acid and fatty acids derivatives are present in both of them. On the other side the study shows that their concentration is higher in the roots and steams. The present study focused on identification of several constituents present in the ethanolic leaves extract of *Brassica oleracea*. This type of GC-MS analysis is the first step towards understanding nature of active compounds in this medicinal plant and helpful for the further detailed study. In this plant contains various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners.

Table 1: Qualitative Phytochemical screening on extracts of *Brassica oleracea*

S. No	Name of Test	Test applied / Reagent used	Leaves extract
1	Alkaloids	A] Mayer's B] Wagner's C] Hagner's D]) Dragndorff's test	+ + + +
2	Flavanoids	HCl and magnesium turnings	+
3	Carbohydrate	Molisch's test	+
4	Tannins & Phenols	A] 10% Lead acetate B] $Fecl_3$	+ +
5	Test for Steroids	A] Salkowski's Test B] Libermann-Burchard's Test	+ +
6	Gums & Mucilages	Alcoholic Precipitation	-
7	Fixed oil & Fats	Spot test	+
8	Saponins	Foam test	-
9	Phytosterols	LB test	+
10	Volatile oils	Hydro distillation method	+
11	Protein & free amino acids.	A] Biuret test B] Ninhydrin test C] Xanthoprotein test	+ + +

-, absents; +, present.

Table 2. GC-MS Analysis of *Brassica oleracea*

S.No	RT	Name of the Compound	Molecular Formula	M.W	Area%
1	15.550	Diethyl Phthalate $$ Diethylphthalat	$C_{12}H_{14}O_4$	222	1.23
2	16.375	Pentadecanal- $$ 1-Pentadecanal $$ n-Pentadecanal $$	$C_{15}H_{30}O$	226	2.05
3	16.642	Oxirane, tetradecyl- $$ Hexadecane, 1,2-epoxy- $$ Hexadecylene oxide $$ 1,2-Epoxyhexadecane $$ 1,2-Hexadecane oxide $$ 1,2-Hexadecene	$C_{16}H_{32}O$	240	3.17
4	16.683	(Z)6-Pentadecen-1-ol $$ (6Z)-6-Pentadecen-1-ol # $$	$C_{15}H_{30}O$	226	2.30
5	16.858	Tetradecanal $$ Myristaldehyde $$ 1-Tetradecanal $$ 1-Tetradecyl Aldehyde $$ -	$C_{14}H_{28}O$	212	1.36
6	18.083	Decanoic Acid $$ Capric Acid $$ Sodium Caprate Sodium Decanoate1-	$C_{10}H_{20}O_2$	172	1.86
7	18.233	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne $$ Neophytadiene	$C_{20}H_{38}$	278	1.93
8	18.317	2-Pentadecanone, 6,10,14-trimethyl- $$ Hexahydrofarnesyl acetone $$ 6,10,14-Trimethyl-2-pentadecanone	$C_{18}H_{36}O$	268	6.87
9	19.175	Isoborneol $$ Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, exo- $$ exo-2-Hydroxy-1,7,7-trimethylnorbornane	$C_{10}H_{18}O$	154	1.56

10	19.408	cis-9-Hexadecenal $$ 9-Hexadecenal, (Z)- $$ (Z)-9-Hexadecenal $$ Z-9-Hexadecenal $$ (9Z)-9-Hexadecenal	$C_{16}H_{30}O$	238	3.71
11	19.558	n-Hexadecanoic acid $$ Hexadecanoic acid $$ n-Hexadecoic acid $$ Palmitic acid $$ Pentadecanecarboxylic acid	$C_{16}H_{32}O_2$	256	25.75
12	19.683	9-Octadecen-1-ol, (Z)- $$ cis-9-Octadecen-1-ol $$ cis-9-Octadecenyl Alcohol $$ Adol 320	$C_{18}H_{36}O$	268	5.42
13	19.742	Z,Z-2,13-Octadecadien-1-ol $$ (2Z,13Z)-2,13-Octadecadien-1-ol	$C_{18}H_{34}O$	266	5.95
14	19.865	Ethyl Pentadecanoate $$ Einecs 255-223-8 $$ N-Pentadecanoic Acid Ethyl Ester	$C_{17}H_{34}O_2$	270	2.04
15	20.575	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	$C_{15}H_{24}$	204	1.07
16	20.850	1-Hexadecanol $$ n-Cetyl alcohol $$ n-Hexadecan-1-ol	$C_{16}H_{34}O$	242	1.31
17	21.192	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl-	$C_{20}H_{40}O$	296	12.20
18	21.542	9,12-Octadecadienoic acid (Z,Z)- $$ cis-9,cis-12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280	13.48
19	21.725	(Z)6-Pentadecen-1-ol $$ (6Z)-6-Pentadecen-1-ol	$C_{15}H_{30}O$	226	4.92
20	21.817	Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{36}O_4$	352	1.81

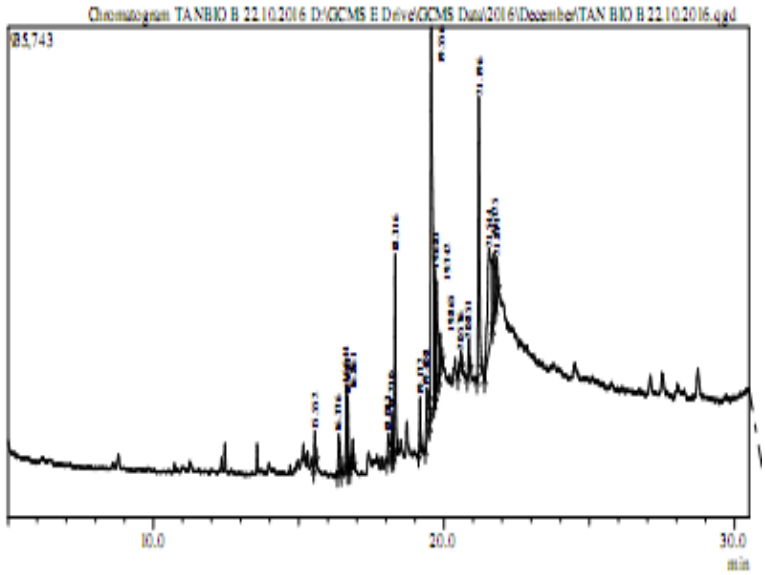

Fig 1. GC-MS Chromatogram of *Brassica oleracea*

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