



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(3): 257-267
Received: 12-07-2015
Accepted: 13-08-2015

Isha Verma
Food Processing Technology
Laboratory, USBT, Guru Gobind
Singh Indraprastha University,
Sector-16 C, Dwarka, New Delhi-
110078, India.

Rajinder K. Gupta
Food Processing Technology
Laboratory, USBT, Guru Gobind
Singh Indraprastha University,
Sector-16 C, Dwarka, New Delhi-
110078, India.

Estimation of phytochemical, nutritional, antioxidant and antibacterial activity of dried fruit of sacred figs (*Ficus religiosa*) and formulation of value added product (Hard Candy)

Isha Verma, Rajinder K Gupta

Abstract

The present study deals with the nutritional, phytochemical, antioxidant and antibacterial activity of dried fruit of sacred figs (*Ficus religiosa* Linn), commonly known as peepal or Bo tree, which belongs to family Moraceae and found throughout India and in other neighboring countries like Nepal, Bangladesh, Myanmar etc. The phytochemical analysis of sacred figs showed the presence of total phenolics, flavanoids, and other secondary metabolites which contributes to its good antioxidant activity which was estimated by FRAP and DPPH scavenging assay, whereas alkaloid content was comparatively low. The nutritional profiling of the dried fruit of sacred fig indicated that it is an admirable source of dietary fiber, proteins, carbohydrates, calcium, and magnesium, phosphorous and relatively negligible amount of fat. This study explains that *Ficus religiosa* figs with high phytochemical and nutritional properties can be utilized as nutraceutical food with highly therapeutic properties.

Further hard boiled candies were prepared using glucose syrup and lemon juice in which dried powder of fruits of *Ficus religiosa* was incorporated along with permitted flavoring and coloring agents for appeal. The sensory evaluation showed very good overall acceptability by the panelists.

Keywords: Sacred figs, phytochemical analysis, Nutritional profiling, hard candy, sensory evaluation.

1. Introduction

The fruits whose majority of water content has been removed either naturally i.e. sun drying or with the help of specialized devices such as dryers or dehydrators are dried fruits. Such fruits like figs, prunes and raisins are a good source of fiber. A fully developed fig having bell or pear shape with succulent flesh inside are naturally rich in health promoting phyto-nutrients, anti-oxidants, concentrated minerals and vitamins.

Ficus religiosa also known as Peepal, Pipal, Ashwattha tree, or Bodhi tree is commonly planted as boulevard and roadside tree^[1]. *Ficus religiosa* is a dry season-deciduous or semi-evergreen tree that goes up to 30 meters (98 ft) tall in height with a trunk of 3 meter diameter in width. It is found throughout the plains of India and up to 170 m altitude in Himalayas. Peepal is most commonly found in temples due to its mythological and religious importance in Indian culture since immemorial times. The popular Bodhi tree is considered sacred by the followers of different religions as Hinduism, Jainism and Buddhism. The fruits of sacred fig are circular in shape and persists small size of about 1-1.5cm in diameter, becomes purple when ripe and unripe ones are green in color, with neutral taste or flavor. *F. religiosa* figs are harvested in the months of May and June but the dried figs are available throughout the year.

1.1. Nutritional and Medicinal attributes

The fruits of *Ficus religiosa* or sacred fig are currently not being marketed commercially but in ayurveda and other medicinal practices it is an efficient medicine prescribed in either dried powdered form for the treatment of asthma, or along with hard for the treatment of diabetes, it is consumed in the fresh form for the treatment of dehydration. It is consumed as famine food during scarcity. According to studies the fruit of sacred figs contains good amounts of β -caryophyllene, α -terpinene tyrosine, undecane, dendrolasine, tridecane, tetradecane, α -trans bergamotene, (e)- β -ocimene, α -pinene, limonene, asgaragine, dendrolasine, α -ylangene, α -thujene, α -copaene, β -bourbonene, aromadendrene, δ -cadinene, α -humulene, β -pinene, alloaromadendrene, germacrene, γ -cadinene and bicyclogermacrene^[2]. As per data provided by various studies, figs of *Ficus religiosa* are an excellent source of flavanoids, phenols, fiber, antioxidants and other compounds like vitamins, proteins, minerals, carbohydrates, serotonin,

Correspondence

Rajinder K. Gupta
Food Processing Technology
Laboratory, USBT, Guru Gobind
Singh Indraprastha University,
Sector-16 C, Dwarka, New Delhi-
110078, India.
Email: rkg67ap@yahoo.com,
Contact No. +91-11-2530321

etc. The high amount of serotonin content showed anticonvulsant activities which were evaluated with the help of Maximum Electroshock model [3] and anti-amnesic activity was observed using a methanol extract of dried *F. religiosa* figs [4]. It has also been reported as an efficient drug for laxative due its high content of fiber.

Due to health benefiting properties the dried powder of sacred fig was incorporated in glucose syrup to make hard candies. It was evaluated for phytochemical, nutritional, antioxidant and antimicrobial activity.

2. Materials and Methods

2.2. Collection and preparation of sample

Fresh figs were collected from *Ficus religiosa* tree located on the Shahbajpur Street of District Budaun, Uttar Pradesh, India. The figs were rinsed with tap water in order to make them clean and dried in hot air oven at 40 °C for 48 hours. The dried figs were then coarsely powdered using a mixer grinder and stored in an airtight container at 4 °C.

2.3. Chemicals and reagents

Aluminium chloride(Fisher), Folin-Ciocalteu's phenol reagent (SRL), Gallic acid, Methanol, Ethanol, TPTZ, Petroleum ether, n-butanol, Sodium chloride, Acetic acid glacial, Sodium hydroxide, Aluminium chloride, Catechin, BHT, Dimethyl sulfoxide(DMSO), 2,4,6 tripyridyl-s-triazine(TPTZ), Nutrient agar, Nutrient broth.

2.4. Material for Candy

White granulated Sugar (Mawana), water, Lemon juice, flavoring and coloring agent and dried powder of sacred figs.

2.5. Preparation of Extract

Solvent extraction was performed using two solvents i.e., methanol and ethanol. 20 gm dried sample powder was added in 200ml of solvent. The mixture was then incubated in an incubator shaker at 60 °C with 150 rpm for 48 hours. The mixture was filtered using Whattman filter paper and the filtrates obtained were evaporated at 60 °C in hot air oven and the dried concentrated extract was stored at 4 °C. The extracts obtained after extraction method was dark green in color.

2.6. Preparation of Candy

Candy was prepared by adding 1 cup of granulated sugar, i.e. 225gm in ¼ cup of water, i.e. 60ml and it was then placed on heat and the dry sample powder with concentration of 23.3 mg/ml was added to the solution. 4 ml lemon juice was added later which is a natural substitute of high fructose corn syrup. Lemon juice prevents the formation of sugar crystals and provides a uniform and translucent appearance to the candy. This solution was heated till the solution reached 146 °C. The solution was then removed from heat to add flavor and coloring agents for appeal. The sugar solution was allowed to cool in order to remove bubbles and then poured into the molds to obtain the desired shape. The hard candy was then wrapped in butter paper and stored at room temperature.

2.7. Nutritional Profiling the nutritional profiling was done to evaluate the qualitative prospects of the dried sample of sacred fig.

2.7.1. The ash content in dry sample was determined by incinerating 2g sample in a Muffle Furnace (MF-1/02, PCSIR, Pakistan) and after charring according to AACC Method 08-01 (AACC, 2000).

2g of the dried powdered sample was taken in preweighed crucible which was then placed uncovered in a muffle furnace at 550 °C to allow charring for 4 hours. The crucible was then taken out, covered with the lid and cooled in a desiccator to avoid any moisture. The crucible was then weighed without the lid using a weighing balance. The total ash content (in %) was calculated by the formula given below-

$$\text{Ash \%} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

2.7.2. The moisture content was determined using the method given by AOAC.

10g of the dried powdered sample was taken in a preweighed and preheated petri plate and was placed in hot air oven at 130 °C to remove any trace of moisture for 2 hours. As soon as the weight of glass petri plates containing sample becomes constant it is placed in a desiccator to cool and then reweighed. The following formula was used to calculate the percentage of total moisture content in the sample:

$$\text{Moisture \%} = \frac{W_1 - W_2}{\text{Weight of the sample}} \times 100$$

Where,

W_1 = Weight of sample before drying (g)

W_2 = Weight of sample after drying (g)

2.7.3. Crude fat content was determined by soaking 5g of the dry powdered sample in 50ml of petroleum ether and this mixture was then incubated in an incubator shaker at 60 °C for 48 hours. The mixture was filtered using whattman filter paper and the filtrate was evaporated at 60 °C. The beaker was then weighed to evaluate the amount of crude fat, which is visible in the form of differentiating layer. The crude Fat content was calculated in percentage using the following formula:

$$\text{Fat \%} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

2.7.4. Total Dietary Fibre & Protein were determined by using the method given by IFS/C/SOP/FC/007/(AOAC 993.21) and IFS/C/SOP/FC/011/(IS-7219-1973) respectively.

2.7.5. Carbohydrates content was calculated by using the following formula:

Total Carbohydrates content % = 100 - [moisture (%) + protein content (%) + crude ash (%) + fat(%)], all data were taken in % fresh weight and the result obtained was in percentage (%).

2.7.6. Energy or calorific value was calculated in kilocalories and was determined by using the following equation:

Kcal = (3.36 × % protein content) + (3.60 × % total carbohydrate content) + (8.37 × % fat)

2.7.7. Minerals and trace elements in the dry powdered sample were evaluated with the help of Optima 2100 DV ICP-OES (Perkin-Elmer, USA), after prior mineralization in an Anton Paar Multiwave digester (Anton Paar Ltd., Hertford, UK) as per Ref 956.52 (AOAC, 2005). The certified multi-element standard solution "ICP Multi-element Standard IV" (Merck, Darmstadt, Germany) was used for the instrument's response^[5].

2.8. Phytochemical analysis

2.8.1. Total phenolic content

Total phenolic content was determined using Folin-Ciocalteu reagent (FC reagent)^[6]. An aliquot (100µl) of extract was mixed with 250µl of FC reagent and was kept at room temperature for 5 min. 1.5ml of 20% sodium bicarbonate was added to this mixture and incubated at room temperature for 2 hours. Optical density was measured at 765 nm using a spectrophotometer. The results were expressed in terms of µg Gallic acid equivalents (GAE)/mg of dry extract.

2.8.2. Total flavonoids content

Total flavonoid content was determined by using aluminium chloride colorimetric method^[7] expressed in terms of Total flavonoids were determined using catechin as standard. The sample extract (250µl) was added to 4.5 ml distilled water, followed by 5% NaNO₂ (0.03ml). After 5 min at 25 °C, AlCl₃ (0.03 ml, 10%) was added. After another 5 min, the reaction mixture was treated with 2 ml of 1M NaOH. Eventually the reaction mixture was diluted to 10 ml with distilled water and absorbance was measured at 510 nm. The results were expressed as catechin equivalents (CE) in µg/mg of dried extract. The results obtained were expressed in terms of catechin equivalents (CE)/g of dry extract.

2.8.3. Crude alkaloid determination

Crude alkaloid content was determined using gravimetric method^[7]. In this method 2.5g of the dry powdered sample was weighed and added in 100ml of 10% acetic acid ethanol. The suspension was incubated for 4 hours at room temperature and later it was filtered using Whatman filter paper and concentrated upto one fourth of its original volume using water bath. Concentrated ammonium hydroxide was added drop wise to obtain precipitate in the solution. This whole solution was then allowed to settle and the precipitate was collected and washed with diluted ammonium hydroxide and then it was filtered using preweighed Whatman filter paper the filter paper was air dried at room temperature and reweighed. The amount of crude alkaloid was determined in percentage by using the following formula:

$$\% \text{ of alkaloid} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Weight of sample}} \times 100$$

2.9. Anti-oxidant Activity

2.9.1. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was determined by the method of Benzie and Strain, 1996^[7]. The FRAP reagent was prepared fresh each day by mixing 2.5 ml of TPTZ (10 mM in 40 mM hydrochloric acid), 2.5 ml of ferric chloride (20 mM) and 25 ml of sodium acetate buffer (300 mM, pH 3.6). A 100 µl of

extract was mixed with 900 µl of FRAP reagent. The mixture was incubated at 37 °C for 4 min. The absorbance was measured at 593 nm and the result was expressed as BHT equivalent.

2.9.2. DPPH radical scavenging activity

DPPH scavenging activity was determined by the method of Blois, 2000^[7] with slight modification. A 10ml extract solution of 50 ppm concentration was prepared by adding 0.5 mg of extracts (Methanol and ethanol) in 10ml of methanol and 1ml of this extract solution was taken in a test tube. Then 1ml of a 0.3 Mm methanol solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was added to each test tube and after this 1ml methanol was also added in each test tube. The solutions were allowed to stand for 10 minutes in dark. Blank was prepared without extract solution. Methanol was used as a reference. Then OD was taken at 517nm.

The radical scavenging activity was expressed in % of scavenging activity and was calculated by the following formula:

Radical Scavenging Activity (%) =

$$\frac{\text{OD Blank} - \text{OD Sample}}{\text{OD Blank}} \times 100$$

2.10. Determination of antimicrobial activity

Antimicrobial activities of the extracts were evaluated by agar well diffusion method against three Gram-positive and two Gram negative bacterial test pathogens^[8].

2.10.1. Test Microorganisms

About five microbial strains were obtained from Microbiology Lab of USBT, GGSIP University namely, *Escherichia coli*, *Salmonella enterica*, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*. The strains were then revived by streaking them on nutrient agar petriplates and the inoculated plates were then incubated at 37 °C for 24 hours in incubator for growth. The revived cultures were then inoculated in nutrient broth contained in test tubes in order to prepare a spreadable media and were then incubated at 37 °C for 24 hours in an incubator shaker to obtain uniform growth. These tubes containing microbial colonies in nutrient broth were then used for the well diffusion method.

2.10.2. Well Diffusion Method

Extracts were reconstituted to final concentrations of 100 mg/ml, 50 mg/ml and 25 mg/ml. Nutrient agar was inoculated by a spread plate method with 100 µl of the 24 hours old bacterial inoculums. Wells (6 mm diameter) were punched using sterile cork borer in the agar and 80 µl of extracts were loaded into the wells. The inoculated plates were then incubated at 37 °C for 24 h. The antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition and reported on the scale of millimeters. Methanol was used as control in one of the wells in each plate.

2.11. Determination of other secondary metabolites

An Agilent 5975B mass spectrometric detector (MSD) was used in the scan mode (m/z 35 – 1050) for the sample.

Screening of volatiles and semi volatiles were performed using the automatic RTL screener software in combination with the Agilent NIST[®] 05 library [5]

2.12. Sensory analysis of product

To establish best product, sensory evaluation was done on 9-point Hedonic scale as described by Larmond (1977) where 1 represents extremely disliked and 9 represents extremely liked.

3. Results and Discussion

3.1. Nutritional Analysis of dried fruit of sacred fig

The Nutritional profile of dried sacred fig has shown its potential health benefits.

3.1.1. Dietary fiber

Nutritional analysis has revealed that dried fruit of sacred fig has dietary fiber as a major component (69.43%) that has a number of health benefits. Soluble dietary fiber lowers total cholesterol, has the ability to relieve or prevent constipation, lowers the risk of diabetes by slowing down the absorption of sugar, and also helps in achieving healthy weight [9, 10].

3.1.2. Protein content

Proteins are macromolecules composed of amino acids which play a vital role in the growth and maintenance in human body [11], whereas amino acids are used in muscle building and for repairing damaged tissues. Proteins are utilized as fuel in lack of carbohydrates and lipid resources [12]. Moderate amount of proteins were found in dried sacred fig i.e., 8.48%.

3.1.3. Moisture content

Dried figs were found to contain 18.8%. Moisture content affects the texture, taste, appearance and stability of foods so it is related to storage attributes of the dried figs.

3.1.4. Ash content

The ash content is a measure of the total amount of minerals present within a food. Total ash content was found to be 4.44%.

3.1.5. Carbohydrates

Carbohydrates are the most abundant and widespread organic substance present in nature. It serves as an immediate source of energy. Although dried sacred figs are not very sweet and persists a neutral taste, it contains a fair amount of carbohydrates which is 68.33%.

3.1.6. Crude Fat content

Dried figs have a very low amount of fat i.e. 0.143%. Therefore, it might be helpful in weight loss.

3.1.7. Energy (Kcal)

The calorific value was calculated using the formula and was found to be 308.52 Kcal. Therefore, it is a good source of energy.

Table 1: Nutritional profile of dried sacred fig fruit

Sample	Dried Fig fruit
Energy (Kcal/100g)	308.52
Total carbohydrate	68.33%
Fat	0.143%
Protein	8.48%
Dietary fiber	69.43%
Moisture	18.8%
Ash	4.44%

3.1.8. Minerals and heavy metals

Minerals provide a wide range of functions for the human body. Different types of mineral show an array of functions which are beneficial. Such as calcium, magnesium, phosphorus provides structure to the bones. An experiment conducted by the United States Department of Agriculture on postmenopausal women indicated that the women who were tested against the definite amount of dose of boron per day, i.e.; 3 mg showed that the auxiliary boron reduced the excretion of calcium by 44% and activated vitamin D showing positive role in the suppression of osteoporosis [13]. However, it could not be determined that whether these effects were conventionally nutritional or medicinal. The mineral content was analyzed by using ICP – OES.

Table 2: Minerals estimated in ICP-OES

Sr. No.	Analyte	Sample concentration Unit(mg/100g)
1.	Aluminium	4.8
2.	Boron	1.6
3.	Barium	1.1
4.	Calcium	848
5.	Copper	0.12
6.	Iron	6
7.	Magnesium	224
8.	Manganese	0.87
9.	Phosphorous	165
10.	Strontium	3.6
11.	Titanium	0.15
12.	Zinc	1.1

3.2. Phytochemical Analysis of Dried Sacred Fig Fruit

The extract was screened for total phenolics, total flavonoids, alkaloids and other secondary metabolites. Polyphenols have the ability to stabilize the unpaired electron and have an ideal structure to prevent harmful oxidation through free radical – scavenging. They have been shown to be more effective antioxidants than vitamin E and C [14, 5]. Flavonoid contributes towards inhibition of cell – proliferation, induction of apoptosis and inhibition of enzymes and also has antibacterial and antioxidant effects [15, 16]. Alkaloids are the active component of many anesthetics, sedatives, stimulants, relaxants and tranquilizers [5]. The crude alkaloid content was calculated on dry weight basis (g/100g). Alkaloid content was found in moderate amount in the figs.

3.2.1. Total Phenolic estimation

The phenolic content was calculated as Gallic acid equivalents (GAE)/g on the basis of standard curve of Gallic acid. The results were expressed as Gallic acid equivalents (GAE)/mg of the sample.

Table 3: Phenolic content evaluated in GA Equivalents

Sample	GA Equivalents (μg GAE/mg sample)
Dried fruit of sacred fig in methanol	10.35
Dried fruit of sacred fig in ethanol	7.33

3.2.2. Total Flavonoid content

The total flavonoid content (TFC) of dried fig extract was determined using the aluminium chloride assay through spectrophotometer. The TFC was expressed in mg of catechin equivalents (CE) per gram of extract.

Table 4: Flavonoid content evaluated in CE Equivalents

Sample	CE Equivalents (μg CE/mg sample)
Dried fruit of sacred fig methanol	43.66
Dried fruit of sacred fig ethanol	42.66

3.2.3. Crude Alkaloid content

As the name depicts alkaloid means alkali like, it binds to nucleic acid of DNA and makes it susceptible to UV damage, effects binding of regulators, and also effects repair. It inhibits phosphodiesterases which is involved in the breakdown of carbohydrates and fat, resulting in accumulation of fat and might even result in obesity. These effects are congenital defects in offspring such as skeletal or palate damage etc. Therefore high amounts of alkaloid are not acceptable as Zygacine from the Death Camus plant induces vomiting, diarrhea, decreased heart rate, gastroenteritis, subnormal temperature [17, 18]. The dried fruit of sacred fig contains negligible amounts of alkaloids i.e. 0.928%. Although the amount of alkaloid which is acceptable for consumption ranges from 0% to 5%.

3.3. Determination of other secondary metabolites by GC – MS

A total of 25 compounds were detected in methanolic and ethanolic extract of dried fruit of *Ficus religiosa* in GC – MS. The main compounds detected by GC – MS in the extract of fig fruit of *Ficus religiosa* were Caryophyllene, Campesterol, Stigmasterol, Beta – Amyrin, Alpha – Amyrin. β – amyryrin and α – amyryrin are found to be very significant as glycyrrhizin is most likely derived from the terpene β -amyryrin, used as natural sweetener [19]. β amyryrin are also found in dandelion coffee [20]. Stigmasterol acts as a precursor in the formation of the semi-synthetic progesterone hormone. [21, 22, 23] It is also used a precursor of vitamin D₃ [24]. It also possesses potent antioxidant, hypoglycemic and thyroid inhibiting properties [25, 26]. Campesterol is found in dandelion coffee (herbal tea) and have anti – inflammatory effects [5]. Campesterol has a number of cosmetics, medical and functional food applications and also provides antioxidant and anti-microbial properties to the fruit. Campesterol having saturated fat reducing and cholesterol lowering properties may reduce the risk of heart diseases [5]. Caryophyllene or β caryophyllene is a constituent of many essential oil. It also contributes to the spiciness of black pepper [5]. A study conducted by the Jürg Gertsch et al. from the Swiss Federal Institute of Technology (ETH Zurich), showed that caryophyllene was selective against cannabinoid receptor type-2 of (CB₂) ad exerted significant cannabimimetic anti-inflammatory effects in mice [27]. Antinociceptive, [28] neuroprotective, [29] anxiolytic and antidepressant [30] and anti-alcoholism [31] activity has also been uncovered.

Table 5: GC-MS analysis of dried sacred fig fruit methanolic extract

S. No	Compound Detected	CAS No.	Retention time	% Area
1.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	028564-83-2	9.771	0.45
2.	Dodecane	000112-40-3	10.657	0.14
3.	Caryophyllene	000087-44-5	14.168	0.08
4.	3-Eicosene, (E)	074685-33-9	16.209	0.60
5.	5-Octadecene, (E)-	007206-21-5	18.474	0.68
6.	n-Hexadecanoic acid	000057-10-3	20.269	10.86
7.	9-Octadecenoic acid (Z)-, methyl ester	000112-62-9	21.547	3.70
8.	Oleic Acid	000112-80-1	21.962	16.48
9.	Eicosanoic acid, methyl ester	001120-28-1	23.521	0.59
10.	Eicosanoic acid	000506-30-9	23.824	0.85
11.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	023470-00-0	25.035	1.35
12.	cis-9-Hexadecenal	056219-04-6	26.437	3.81
13.	1-Docosene	001599-67-3	28.254	3.62
14.	9-Nonadecene	031035-07-1	30.508	1.62
15.	13-Tetradecen-1-ol acetate	056221-91-1	30.879	0.85
14.	Cholest-5-en-3-ol (3. beta.)-	000057-88-5	31.473	1.40
17.	Campesterol	000474-62-4	33.413	1.36

18.	Stigmasterol	000083-48-7	34.086	3.68
19.	Stigmasterol, 22,23-dihydro-	1000214-20-7	35.387	4.17
20.	beta.-Amyrin	000559-70-6	36.195	2.42
21.	4,4,6a,6b,8a,11,12,14b-Octamethyl,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b octadecahydro-2H-picen-3-one	1000194-64-2	36.666	1.31
22.	alpha.-Amyrin	000638-95-9	37.383	5.21
23.	12-Oleanen-3-yl acetate, (3.alpha.)	033055-28-6	38.976	4.97
24.	Urs-12-en-24-oic acid, 3-oxo-,methyl ester,	020475-86-9	40.378	10.93
25.	1-Tetradecene	001120-36-1	13.618	0.36
26.	1-Hexadecene	000629-73-2	16.198	0.60

Table 6: GC-MS analysis of dried sacred fig fruit ethanolic extract

S. No.	Compounds detected	CAS No.	Retention Time	% Area
1.	1-Tetradecene	001120-36-1	13.618	0.36
2.	alpha.-Caryophyllene	006753-98-6	14.628	0.15
3.	1-Hexadecene	000629-73-2	16.198	0.60
4.	Tetradecanoic acid	000544-63-8	18.149	0.23
5.	1-Octadecene	000112-88-9	18.463	0.55
6.	Pentadecanoic acid	001002-84-2	19.192	0.14
7.	n-Hexadecanoic acid	000057-10-3	20.280	11.04
8.	Heptadecanoic acid	000506-12-7	21.177	0.32
9.	Octadecanoic acid, ethyl ester	000111-61-5	22.366	1.62
10.	Cyclotetracosane	000297-03-0	24.060	0.72
11.	2,3-Dihydroxypropyl elaidate	002716-53-2	26.426	3.71
12.	Tetracosane	000646-31-1	28.254	4.76
13.	9-Nonadecene	031035-07-1	30.520	2.23
14.	Eicosane	000112-95-8	30.822	1.31
15.	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta [a]phenanthren-3-ol	1000210-38-4	31.473	1.88
16.	Campesterol	000474-62-4	33.413	1.63
17.	Stigmasterol	000083-48-7	34.086	4.53
18.	Stigmasterol,22,23-dihydro-	1000214-20-7	35.398	4.16
19.	beta.-Amyrin	000559-70-6	36.195	2.58
20.	4,4,6a,6b,8a,11,12,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	1000194-64-2	36.688	1.05
21.	alpha.-Amyrin	000638-95-9	37.395	5.34
22.	12-Oleanen-3-yl acetate,(3.alpha.)	033055-28-6	38.998	5.44
23.	Urs-12-en-24-oic acid, 3-oxo-, methyl ester	020475-86-9	40.423	12.23

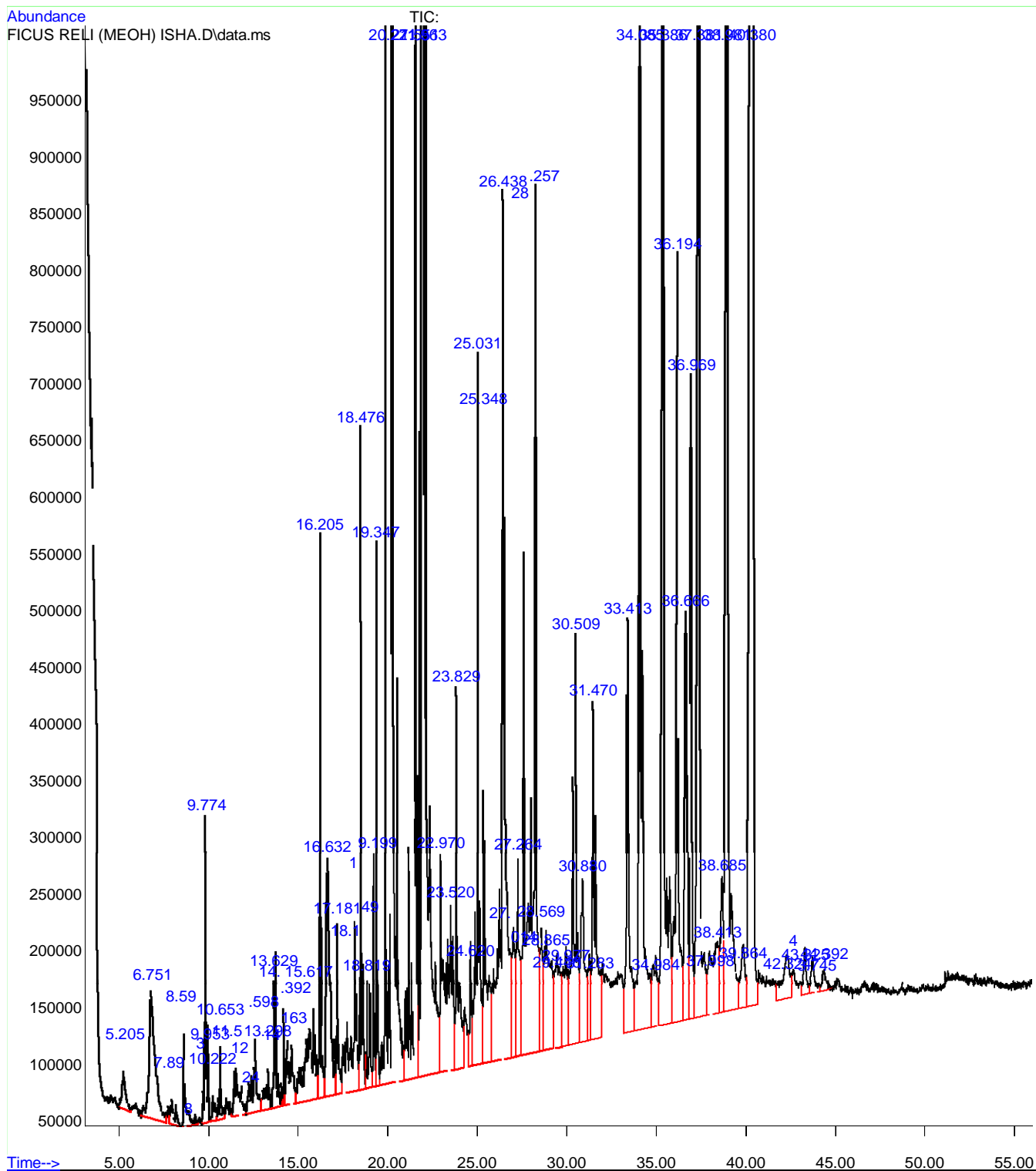


Fig1 (A): GC-MS chromatogram of methanolic extract of figs (*Ficus religiosa*)

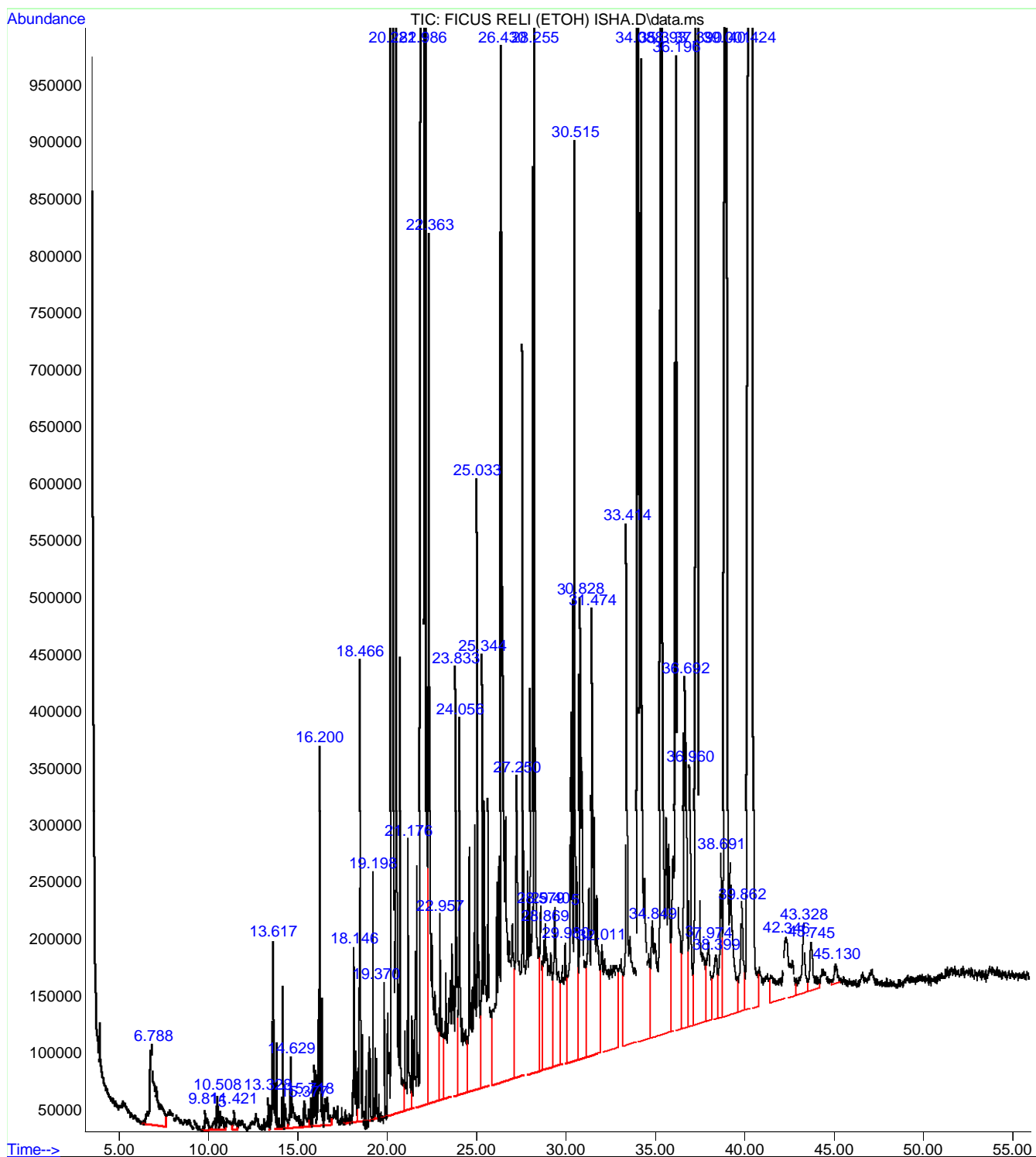


Fig 1 (B): GC-MS chromatogram of ethanolic extract of figs (*Ficus religiosa*)

3.4. Antioxidant activity

3.4.1. Ferric Reducing Antioxidant Power (FRAP)

The anti-oxidant potential of dried fruit extract of sacred fig was determined against BHT by using Ferric reducing anti-oxidant power. In this method reduction of ferric-tripyridyltriazine to ferrous complex forms an intense blue

color. The intensity of the color acts an indicator of the amount of antioxidant reductants in the sample. FRAP activity in the sample was found to be prominent.

Table 7: Antioxidant activity estimated by FRAP

Sample	BHT Equivalents (μg BHT/mg sample)
Dried fruit of sacred fig in methanol	197.65
Dried fruit of sacred fig in ethanol	194.20

3.4.2. DPPH radical Scavenging Activity

The anti-oxidant activity was also calculated against free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The DPPH radical is widely used to evaluate the scavenging activities of various natural compounds such as anthocyanins, crude extracts of plants or phenolic compounds. The mechanism of this model system shows that the antioxidants present in the compound scavenge the DPPH radical by donating a hydrogen ion, forming the reduced DPPH-H•. Due to reduction the colour of the solution changes from purple to yellow which is quantified by its decrease of absorbance at wavelength 517 nm^[32]. It was found in 0.05mg/ml of ethanolic extract of dried fruit of sacred fig had the highest scavenging activity, i.e. 94.40%, followed by a methanolic extract of dried fruit of sacred fig which is 91.24%.

3.5. Determination of Antimicrobial Activity

The antimicrobial potential of both the experimental fruit extract was evaluated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were compared with the activity of the standard, methanol^[33]. For all the tested microorganisms such as *Bacillus cereus*, *Bacillus subtilis*, *E.coli*, *Salmonella enterica*, and *Staphylococcus aureus*, Ethanol extract of dried fruit of sacred fig showed maximum antibacterial activity against *Escherichia coli* and *Bacillus aureus* and methanol extract of same sample showed maximum activity against *Bacillus cereus*. The table below depicts the results of antimicrobial activity against different concentrations of samples in tabular form.

Table 8: Zone of Inhibition against test microorganisms showing Antimicrobial activity

S. no.	Test Microorganism	Extract	Extract Conc.	Zone of Inhibition (mm)
1.	<i>Escherichia coli</i>	Ethanol	100mg/ml	12
			50mg/ml	14
			25mg/ml	11
2.	<i>Bacillus cereus</i>	Methanol	50mg/ml	12
3.	<i>Bacillus aureus</i>	Ethanol	25mg/ml	18

3.6. Nutritional and physical attribute of the product-

Product	Moisture (in %)	Calorie Content (cal)	Weight (gm)
Value added Hard Candy	6	72	18.4

**Fig 2:** Value added product (Hard Candy)**Table 9:** Sensory Evaluation of product

Panelists	Color	Taste	Hardness	Sweetness	Flavor	Appearance	Overall acceptance
1.	8	8	8.5	8	7.5	8.5	8
2.	9	8.5	8.5	7.5	8	8.5	8
3.	8.5	8	8	8	8	8.5	8
4.	8	7	8.5	9	7.5	8.5	7
5.	8.5	8	8.5	8.5	8	8.5	8
6.	9	6	8	7	7	9	7.5
7.	9	8	9	8.5	8.5	9	8
8.	8	7.5	9	8	8	7	8

4. Conclusion

The dried fruits of sacred figs were analyzed for their nutritional, phytochemical, antioxidant and antibacterial activity for its health benefiting properties and its potential to be used as a functional food. The phytochemistry of sacred figs showed the presence of bioactive compounds such as phenols, flavonoids in good amounts which depict their potential to be utilized in traditional medicinal practices. The fruits are rich in dietary fiber, carbohydrates, protein, and minerals such as calcium, phosphorus, iron and magnesium which is evident that it can be used for the formulation of value added products drug development and supplements. The

antioxidant and antimicrobial activity was found to be good. Due to the above properties of dried sacred fig it was formulated in value added product hard candy, which accumulates the therapeutic and health benefiting properties of sacred figs and was checked for sensory evaluation, which proved to be good in appeal, taste, aroma and was overall accepted by the panelists.

5. Acknowledgement

We are highly grateful to the University Grant Commission for the financial support under the Special Assistance Program (SAP) from 2011-2016

6. References

- Ministry of health and family welfare, department of Ayush. New Delhi: 2001. Ayurvedic pharmacopeia of India; 17–20.
- Makhija Kumar Inder, Sharma Indra Prakash, Khamar Devang. Phytochemistry and Pharmacological properties of *Ficus religiosa*: an overview” Annals of Biological Research, 2010;1(4):171-180.
- Singh D, Goel RK J. Anticonvulsant effect of *Ficus religiosa*: role of serotonergic pathways”, J Ethnopharmacol. 2009; 22: 123(2):330-4. doi: 10.1016/j.jep.2009.02.042. Epub 2009 Mar 9
- Kaur H, Singh D, Singh B, Goel RK. Pharmaceutical Biology. 2010; 48(2):234–240.
- Soni Neha, Mehta Sanchi, Satpathy Gouri, Gupta Rajinder K. Estimation of nutritional, phytochemical, antioxidant and antibacterial activity of dried fig (*Ficus carica*), Journal of Pharmacognosy and Phytochemistry. 2014; 3(2):158-165.
- Mcdonald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extract. Food Chemistry. 2001; 73:73-84.
- Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A, Bekhradnia AR. Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica* Mey. *Pharmacologyonline*. 2008; 2:560-567.
- Herborne JB. Phytochemical Methods. Chapman and Hall, London, 1973.
- Duyff RL. American Dietetic Association Complete Food and Nutrition Guide. 4th ed. Hoboken, N.J.: John Wiley & Sons; 2012, 55.
- Slavin JL. Position of the American Dietetic Association: Health implications of dietary fiber. Journal of the American Dietetic Association. 2008; 108:1716.
- Hermann, Janice R. Protein and the Body. Oklahoma Cooperative Extension Service, Division of Agricultural Sciences and Natural Resources, Oklahoma State University: T-3163-1 – T-3163-4
- Nutrition Working Group of the International Olympic Committee (2003). IOC Consensus Conference on Nutrition for Sport. Lausanne.
- Mahler RL. Essential Plant Micronutrients. Boron in Idaho. University of Idaho. Co-operative Extension System Agricultural Experiment Station. CIS 1085
- Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends in Plant Science” Trends in Plant Science. 1997; 2:152 – 159.
- Cook NC, Samman S. Flavonoids – chemistry, metabolism, cardioprotective effects, and dietary sources. Journal of Nutritional Biochemistry. 1996; 7:66-76.
- Middleton E, Kandaswami C. Effects of flavonoids on immune and inflammatory cell function. Biochemical Pharmacology. 1992; 43:1167-1179.
- Saxton JE. The Alkaloids. A Specialist Periodical Report. London”. The Chemical Society. 1971.
- Hesse, Manfred “Alkaloids: Nature's Curse or Blessing?”. Wiley-VCH 2002. ISBN 978-3-906390-24-6.
- Seki H, Ohyama K, Sawai S, Mizutzzni M, Ohnishi T, Sudo H. “Licorice β -amyirin 11-oxidase, a cytochrome P450 with a key role in the biosynthesis of the triterpene sweetner glycyrrhizin”. Proceedings of the national Academy of Sciences USA. 2008; 105(37):14204– 4209.
- Merck Index, 11th Edition, 653.
- Sundararaman P, Djerassi C. A convenient synthesis of progesterone from stigmasterol. J Org Chem 1977; 42 (22):3633–3634. doi:10.1021/jo00442a044. PMID 915584
- Nova Transcripts: Forgotten Genius. pbs.org. February 6, 2007.
- Giants of the Past. lipidlibrary.aocs.org
- Kametani T, Furuyama H. (1987). Synthesis of vitamin D3 and related compounds. Medicinal Research Reviews. 1987; 7(2): 147–171. doi:10.1002/med.2610070202. PMID 3033409.
- Panda S, Jafri M, Kar A, Meheta BK. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. *Fitoterapia* 2009; 80 (2):123.26. doi:10.1016/j.fitote.2008.12.002. PMID19105977.
- Gabay O, Sanchez C, Chevy F, Breton M, Nourissat G. Stigmasterol: A phytosterol with potential anti-osteoarthritic properties. The American Journal of Clinical Nutrition. 2010; 18(1):106–116.
- Gertsch J, Leonti M, Raduner S. Beta-caryophyllene is a dietary cannabinoid". Proceedings of the National Academy of Sciences of the United States of America. 2008. 105 (26): 9099–104. doi:10.1073/pnas.0803601105. PMC 2449371. PMID 185 74142.
- Katsuyama S, Mizoguchi H, Kuwahata H, *et al.* "Involvement of peripheral cannabinoid and opioid receptors in β -caryophyllene-induced antinociception. European Journal of Pain. 2013; 17(5):664–675. doi:10.1002/j.1532-2149.2012.00242.x.
- Guimarães-Santos, Adriano. "Copaiba Oil-Resin Treatment Is Neuroprotective and Reduces Neutrophil Recruitment and Microglia Activation after Motor Cortex Excitotoxic Injury". Evidence-Based Complementary and Alternative Medicine, 2012, 1–9. doi:10.1155/2012/918174. PMC 3291111.
- Bahi Amine, Al Mansouri Shamma, Al Memari Elyazia, Al Ameri Mouza, Nurulain Syed M., Ojha Shreesh. " β -Caryophyllene, a CB2 receptor agonist produces multiple behavioral changes relevant to anxiety and depression in mice.". *Physiology & Behavior* June 2014. 135: 119–124. doi:10.1016/j.physbeh.2014.06.003.
- Al Mansouri Shamma, Ojha Shreesh, Al Maamari Elyazia, Al Ameri Mouza, Nurulain Syed M., Bahi Amine. The cannabinoid receptor 2 agonist, β -caryophyllene, reduced voluntary alcohol intake and attenuated ethanol-induced place preference and sensitivity in mice. *Pharmacology, Biochemistry, and Behavior* 2014; 124C:260-268 124: 260–268. doi:10.1016/j.pbb.2014.06.025.
- Heng-Yuan CHANG, Yu-Ling HO, Ming-Jyh SHEU, Yaw-Huei LIN, Mu-Chuan TSENG, Sheng-Hua WU, *et al.* “Antioxidant and free radical scavenging activities of *Phellinus merrillii* extracts”. Botanical Studies. 2007; 48:407-417.

33. Sen Amrita, Batra Amla. Evaluation of Antimicrobial Activity of Different Solvent Extract of Medicinal Plant: *Melia azedarach* L. International Journal of Current Pharmaceutical Research. 2012; 4:2. ISSN-0975-7066.