ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of Phytochemical and Pharmacological Properties of *Cichorium intybus* (L) Based on Supercritical Fluid Extract.

Ola Basa'ar[1], Samreen Fatema[1], Mohammed Mohsin[1], and Mazahar Farooqui*[1,2].

[1]Post graduate & Research Center, Maulana azad College, Aurangabad (M.S), India.
[2]Dr. Rafiq Zakaria College For Women, Aurangabad (M.S), India.

ABSTRACT

The phytochemical profiles of four types of extracts of *Cichorium Intybus (L)* roots are examined and evaluated. This roots are extracted by different methods, some of them are traditional like decoction method and soxhlet extraction. Supercritical fluid extraction (SFE) also is used as a modern method. The fourth type of extract is extracted by pure methanol which used as a co-solvent with CO_2 in SFE. The qualitative screening for several phytochemical compounds by different tests which revealed the availability of many important phytochemical compounds such as carbohydrates, alkaloids, glycosides, phytosterols, amino acids ,phenols ,flavonoids, fixed oil and fats. Furthermore the pharmacological activities are tested for the SC-CO_2 extract to examine anti-tuberculosis, anti-malaria and anti-microbial activities because it is not found any related study regarding the phytochemical and Pharmacological analysis of such type of extract.

Keyword: Phytochemical, SFE, Anti-Microbial, Anti-Malaria, Anti-Tuberculosis

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Cichorium Intybus (L) is one of the familiar plants which have been used to treat different health problems in traditional medicine. It is named as Kasani, Munnchatti, Hinduba,Wild Succor or Chicory. It is an aromatic biennial plant with lavender flower and it was considered as a member of composite family [1]. This important medicinal herb usually found beside the roadside and meadows and it grows like a flower or a weed [2]**.** The dried roots of chicory are used like a substitute in coffee powder [3] whereas its young leaves can be used in salads and vegetable dishes chicory is a woody herbaceous plant that is very useful in the field of medicine. It has many uses in different ways. Some of its important health benefits are the ability to ease digestive problems, reduce the pains of arthritis, prevent heart burn, detoxify the liver and gallbladder, prevent bacterial infection, it can reduce the chance to hearth diseases, it can protect against kidney stones and it has a benefit to lose weight, therefore this small plant consider as a powerful addition to any diet. Chicory also has a nutritive value, since it is a great source of vitamins such as vitamin A, B_6, C,E and K and minerals including zinc, magnesium, calcium, manganese, iron folic acid and potassium. The extract of whole plant is found to be anti-oxidant [4, 5], anti-hepatotoxic [6,7], anti-diabetic [8] and anti-bacterial [9]. The roots of chicory which are highlighted in this study have been applied since the 17 century for treatment of some diseases such as liver disorders, inflammations of the urinary tract and gall stone [1]. Further it has been used in traditional medicine like Unani and Ayurvedic systems [10].Leaves and roots of Chicorium Intybus L. are further applied for purification of blood or curing anteriosclerosis and they are also regarded as anti-artritis, anti-spasmodic, hypotensive, and laxative action [11].

In the current study the extracts obtained by using different solvents for different methods. Traditional methods such as decoction method is conducted by using distilled water as a solvent, soxhlet extraction is done by using di ethyl ether as a solvent. Additionally supercritical fluid extraction as a modern method is applied by using carbon dioxide as a solvent and pure methanol as a co-solvent, furthermore one type of the extract produced as a result of passing the methanol only in supercritical fluid extraction which consider as the first step in the extraction by this method. Supercritical fluid extraction has become an important common method for the extraction of various organometallic and inorganic compounds in which a supercritical fluid is used as the extracting solvent. Any fluid that exists at a temperature and pressure above its critical point is known as supercritical fluid. The solvating strength of a supercritical fluid is related to the density directly which can be varied by controlling the pressure and temperature. The densities and solvating properties like the liquid solvents, but they possess quick diffusion and viscosities like gases. Carbon dioxide is the most popular supercritical fluid due to many advantages like its low critical temperature (31.06 ℃) and pressure (37 atm).

It is also non-toxic, non- flammable, inert to most compounds and available with a high degree of purity at less cost. Also, it can be removed simply from the solute after extraction is done, leaving behind no chemical residue, which can be a problem in traditional extraction methods.

In addition SFE can be conducted in the absence of oxygen and light so the chances of oxidative degradation of phytocompounds are reduced [12].

These four types of the extracts are compared for their biologically active components and because there is uncompleted information about the biological activities exhibited by the extract of chicory roots produced by the modern method, supercritical fluid extraction technology, it is highlighted in this study. Three important biological activities are tested anti-tuberculosis, anti-malaria and anti-microbial activities.

MATERIALS AND METHODS

The roots of *Cichorium Intybus (L)* is subjected to various steps regarding the extraction process and analysis the extracts. Figure (1) illustrates the flowchart of the experimental processes.

ISSN: 0975-8585

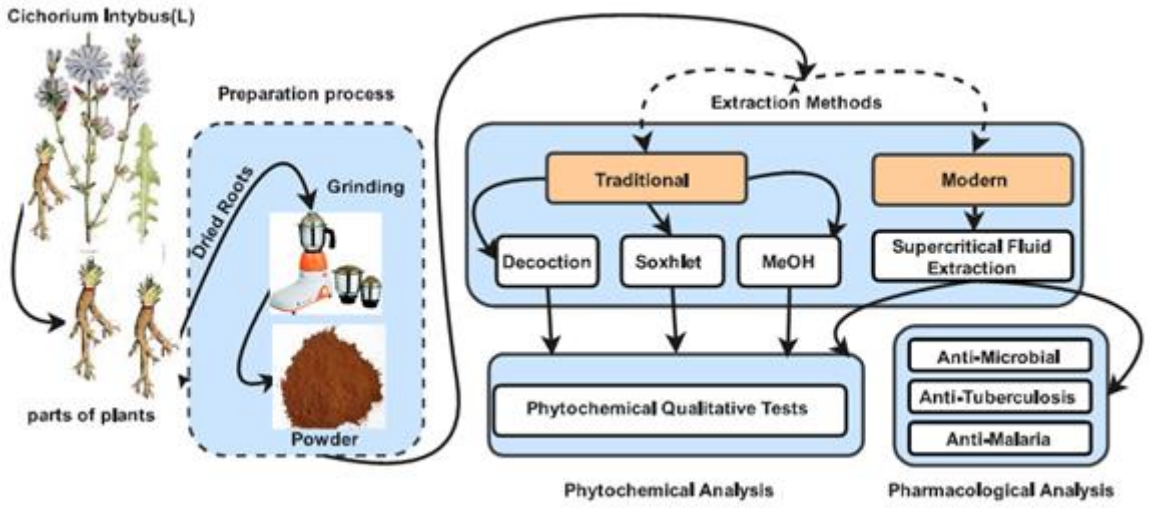

Figure 1. Flowchart of Experimental process

Materials

The dried roots of *Cichorium Intybus (L)* which is available in the market of Aurangabad City, India are taken. All solvents used are of analytical grade and purchased from Sigma-Aldrich Chemicals Co., India. Carbon dioxide with a purity of 99.95 % is brought and used for SFE.

Preparation of the sample

The dried roots of *Cichorium Intybus (L)* are ground properly in electric mixer and kept in air tight glass container for extraction purposes.

Extraction methods

The roots of *Cichorium Intybus (L)* are extracted using both conventional methods like Decoction method, Soxhlet Extraction (SE) and MeOH Extraction. In addition Supercritical Fluid Extraction (SFE) as a recent method is used as reported in our previous work [13].

Qualitative phytochemical screening Experiment

The qualitative phytochemical profile of every type of the solvent free extract of *Cichorium Intybus (L)* roots are established and reported by performing a variety of qualitative chemical testes. Carbohydrates are detected by Molish's test, Benedict's test and Fehling's test. To detect the presence of alkaloids, about 50 mg of dried extract is dissolved in 5 ml of dilute hydrochloric acid and filtered. The filtrate is then examined using Hager's test, Mayer's test, Wagner's test and Dragendorff's test. To study the presence of glycosides, about 50 mg of the extract is hydrolyzed with concentrated hydrochloric acid for 2 hours on water bath, then filtrated and the hydrolysate is subjected to the Borntrager's test and Modified Borntrager's test in addition the extract examined by using Legal's test and keller killiani test. The saponins in the extracts are detected by Froth test and Foam test. The salkowski's test is used to detect the phytosterols. Phenols present in the extract are detected by using Ferric chloride test. To examine the presence of tannins, three different tests are carried out i.e. Gelatin test, Braemer's test and Potassium Dichromate test. To detect the availability of flavonoids, alkaline reagent test, lead acetate test in addition to magnesium and hydrochloric acid reduction test are applied. Detection of proteins and amino acids are carried out by dissolving about 100 mg of the dried extract in 10 ml of distilled water and filtered through Whatman no.1 filter paper, the filtrate then subjected to Biuret test and precipitation by concentrated salt solution to detect the proteins, Ninhydrin test and xanthoproteic test to detect the amino acids. Fixed oils and fats are detected by using spot test and saponification test. The availability of gum and mucilage, about 100 mg of the extract is dissolved in 10 ml of distilled water and

treated with 25 ml of absolute alcohol with constant stirring. Formation of white or cloudy precipitate indicates a positive result for the test. The details of the mention tests are given in [34, 35].

Pharmacological Experiments

A proper review of literature have exhibited that, there are no data available on the pharmacological properties of *Cichorium Intybus(L)* extracted by SFE, which give this study a great value in the field of medicinal plants.

Study of Anti-Microbial Activity

The Broth Dilution Method is used to evaluate the antibacterial activity. The Minimal Inhibition Concentration (MIC) is determined by this method and the methods used for primary and secondary screening as follow: Each synthesized drug is diluted obtaining 2000 microgram /ml concentration, as a stock solution. In primary screening 1000 micro/ml, 500 micro/ml, and 250 micro/ml concentrations of the synthesized drugs are taken. The active synthesized drugs found in this primary screening are further tested in a second set of dilution against all microorganisms. In secondary screening the drugs found active in primary screening are similarly diluted to obtain 200 micro/ml 100 micro/ml, 50 micro/ml, 25 micro/ml, 12.5 micro/ml, 6.250 micro/ml, and concentrations. The highest dilution showing at least 99 % inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculum. The test mixture should contain 108 organism/ml.

Study of Anti-Tuberculosis Activity

The slope method is used to evaluate the anti-tuberculosis activity in which the minimal inhibition concentration technique is used. The extract drug is diluted obtaining 2000 microgram /ml concentration, as a stock solution. In primary screening 500 micro/ml, 250 micro/ml, and 125 micro/ml concentrations of the synthesized drugs are taken. The active synthesized drugs found in this primary screening are further tested in a second set of dilution against all microorganisms. In secondary screening the drugs found active in primary screening are similarly diluted to obtain 100 micro/ml, 50 micro/ml, 25 micro/ml, 12.5 micro/ml, 6.250 micro/ml, 3.125 micro/ml and 1.5625 micro/ml concentrations. The highest dilution showing at least 99 % inhibition is taken as MIC. The result of this is much affected by the size of the inoculum. The test mixture should contain 108 organism/ml. The Standard strain M.tuberculosis, H37 RV is tested with each new batch of medium. The recommended drug concentrations are 4 mg/l for streptomycin, 0.2 mg/l for isoniazide, 40 mg/l for Rifampicin and 2 mg/ l for ethambutol.

Study of Anti-Malaria Activity

The in vitro antimalarial assay is carried out in 96 well microtitre plates according to the microassay protocol of Rieckmann and co-workers with minor modifications. The cultures of P. falciparum strain are maintained in medium RPMI 1640 supplemented with 25mMHE PES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites of P. falciparum are synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitaemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200 µ l of medium RPMI-1640 is determined by Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitaemia (rings) and uniformly maintained with 50% RBCs (O+). A stock solution of 5mg/ml of each of the test samples is prepared in DMSO and subsequent dilutions are prepared with culture medium. The diluted samples in 20 µ l volume are added to the test wells so as to obtain final concentrations (at five- fold dilutions) ranging between 0.4 µ g/ml to 100 µ g/ml in duplicate well containing parasitized cell preparation. The culture plates are incubated at 37 ^{0}C in a candle jar. After 36 to 40 h incubation, thin blood smears from each well are prepared and stained with JSB stain. The slides are microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts is recorded as the minimum inhibitory concentrations (MIC). Chloroquine is used as the reference drug. The mean number of rings, trophozoites and schizonts recorded per 100 parasites from duplicate wells after incubation for 38 hours, and percent maturation inhibition with respect to control group.

ISSN: 0975-8585

RESULT AND DISCUSSION

Qualitative phytochemical Analysis

The extracts of *Cichorium Intybus (L)* roots exhibited a wide range of phytochemical compounds which are proved by the several tests conducted on every extract. All the extracts highlighted in this study reported to possess some similar of phytochemical components such as carbohydrates , alkaloids, phytosterols, fixed oils and fats but their percentage expected to be different. Carbohydrates were found to be the essential constituents of several natural products which considered as important source of herbal medicine. Carbohydrates have many benefits such increase the solubility of drugs in water, lower toxicity and decided to be the responsible for the bioactivities of the natural drugs. They may exist as free monosaccharaides, oligosachharides, polysaccharides and as essential constituents of glucoconjugated, including glucolipids, glycoproteins and glycosylated. The naturally occurring glycosylated were familiar in their antimicrobial drugs and recently as anti-cancer agents [14]. Alkaloids were known in traditional medicine many years ago. Many research studies revealed their biological activities such as cytoxicity [15, 16], analgesic [16, 17, 18], anti-spasmodic and anti-bacterial [16, 19, 20]. Phytosterols are cholesterol like molecules, they inhibit the absorption of regular dietary cholesterol and themselves are not absorbed easily hence decrease the cholesterol level resulting in reducing risk for heart diseases [21, 22, 23]. Furthermore many studies showed that phytosterols can fight cancer cells by stopping the growth and spread of cells that already presented. It is detected the prevention of phytostrols against ovarian, breast, stomach and lung cancer [22, 24]. Skin care is also considered as another important benefit of phytostrols by stopped the slow-down of production of collagen and encouraged new collagen production [24, 25, 26]. The fixed oils and fats are water –insoluble substances of plant. The main constituents of these simple lipids are triglycerides .Fats normally solid or semisolid at room temperature while oils are liquid. They also consist of a low proportion of another lypophilic compounds such as fatty acids, fatty alcohols, vitamins and phytosterols. Waxes are known as esters produced by the combination of fatty acids with monohydroxy alcohols of high molecular weight. These components were used widely in the pharmaceutical and cosmetical fields. Recently many studies revealed the importance of such compounds to health in diagnostic and their uses as medicines in addition to their antioxidant properties [26]. Aqueous extract and MeOH extract are the rich extracts with the phytochemical compounds. Phenols and tannins compounds are found only in aqueous extract based on the tests which are done but flavonoids are detected in aqueous and MeOH extracts. Several biological activities mainly anti-aging, anti-carcinogen and anti-inflammation were detected in the plant which has phenolic compounds. The phenolic compounds such as flavonoids, phenolic acids, tocopherols, etc.are considered as natural anti-oxidants[16,27], therefore the anti-oxidant activity is detected in the plants which possess this type of compounds[16,21,22].Tannins are water soluble polyphenols of high molecular weight. The antimicrobial properties of tannins are explained properly. The growth of many bacteria, fungus, yeasts and viruses is prevented by tannins. In addition anti-carcinogenic and anti-mutagenic activities were reported. These activities related to their antioxidant activity .Flavonoids are hydroxylated phenolic compounds synthesized by plant due to microbial infection and they showed their antimicrobial activity against many types of microorganisms in vitro. The reason behind that is their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [16, 28]. Amino acids also found in aqueous and MeOH extracts. Amino acids known as the basic structural and functional units of proteins they are responsible for the building block of proteins and they act as intermediates in the metabolism of secondary metabolites thus amino acids play significant role in the herbal drugs[29].Cardiac glycosides especially cardenolides glycosides are detected in(SE) and (MeOH) extracts which are responsiple for legal's test and digital glycoside which is detected in MeOH extract and is responsible for Killer Kiliani's test. These compounds are known for lowering the blood pressure as mention in number of studies [30]. The cardiac glycoside which detected in MeOH extract by Keller Killani test consider as a group of steroids and they act as cardio tonic agents .They exhibit high specific action on cardiac muscle, increasing tone, excitability and contractility of this muscle, therefore the weakened heart function becomes more efficient. The important cardiac glycoside which used as a drug to treat the heart failure called digoxin (23) and some new research depicted the anticancer activity for it [31, 32, 33]. Table (1) explained the different tests applied to detect variety of phytochemical constituents for every type of the extracts.

ISSN: 0975-8585

Table 1: Phytochemical Constituents of Chicory Roots Extracts

Sr.No	Chemical constituents	Aqueous Extract	S.E Extract	MeOH Extract	SC-CO_2 Extract
1	**Test for Carbohydrates**				
	A. Molish's test	+	-	+	+
	B. Bendict's test	+	+	+	+
	C. Fehling test	+	+	+	+
2	**Test for alkaloids**				
	A. Hager's test	+	+	+	+
	B. Mayer's test	+	-	-	+
	C. Wagner's test	+	-	-	-
	D. Dragendroff's test	+	-	-	-
3	**Test for glycosides**				
	A. Borntrager's test	-	-	-	-
	B. Legal's test	-	+	+	-
	C. Keller Killani test	-	-	+	-
	D. Modeified Borntrager's test	-	-	-	-
4	**Test for saponins**				
	A. Froth test	-	-	-	-
	B. Foam test	-	-	-	-
5	**Test for Phytosterols**				
	A. Salkowski's test	+	+	+	+
6	**Test for phenols**				
	A. Ferric chloride test	+	-	-	-
7	**Test for tannins**				
	A. Gelatin test	+ + +	-	-	-
	B. Bramer's test	+ + +	-	-	-
	C. Potassium di-chromate test	+	-	-	-
8	**Test for flavonides**				
	A. Alkaline test	+	-	+	-
	B. Lead acetate test	+	-	+	-
	C. Mg &HCL reduction test	-	-	-	-
9	**Test for amino acids**				
	A. Ninhydrin test	+	-	+	-
	B. Xanthoproteic test	+	-	+	-
10	**Test for proteins**				
	A. Buriet test	-	-	-	-
	B. Precipitation by alkolaid reagent test	-	-	-	-
11	**Test for fixed oils & fats**				
	A. Spot test	+ +	+ + +	+ + +	+ + +
	B. Saponification test	+ +	+ + +	+ + +	+ + +
12	**Test for Gum & Mucilage**	-	-	-	-

Where, + = indicates presence of phytochemicals and - = indicates absence of phytochemicals., ++ = shows moderate concentration.
+++ = shows high concentration.

ISSN: 0975-8585

Pharmacological analysis

Study of Anti-Microbial Activity

The results show that the SC-CO_2 extract has anti-microbial activity against gram negative, gram positive and fungus tested .The extract obtained by SFE inhibited the growth of E.Coli ,P.Aeruginose (gram negative) with the lowest tested concentration 100 µg/ml and 125 µg/ml respectively. Four standard drugs are used for comparison (Gentamycin, Chloramphenicol, Ciprofloxacin and Norfloxacin). Their minimal bactericidal concentrations(MBC)are (0.05,50,25,10) µg/ml respectively in case of E.Coli and (1,50,25,10)µ/ml respectively in case of P.Aeruginose. Hence the extract showed moderate inhibition compared with standard drug (Chloramphenicol) and less inhibition compared with the other standard drugs used. Further the SC-CO_2 extract is inhibited the growth of S.Aureus and S.Pyogenus(gram positive)with the lowest tested concentration 200 µg/ml and 100 µg/ml . The standard drugs used gave MBC of (0.25, 50, 50, 10) µg/ml respectively with S.Aureus and (0.5, 50, 50, 10) µg/ml respectively in case of S.Pyogenus. Therefore the extract considered to have moderate inhibition comparing with two given standard drugs (Chloramphenicol and Ciprofloxacin) and less inhibition on this two wild strains than the other standard drugs. Table 2 explained the MIC values of the extract and MBC values of the standard drugs for every type of bacteria used, and figure (2) represents comparison between these values. A previous study[34] tested the anti-bacterial activity of the chicory roots using the agar well diffusion method by measuring the diameter of growth inhibition zones with 50 and 100 µl of five solvent extracts(petroleum ether , chloroform, hexane, ethyl acetate and water).The standard drug used is chloramphenicol. The result reported that the maximum inhibition is detected by the hexane extract at 100 µl against the tested gram positive (B.subtilis, S.Aureus and micrococcus luteus) and gram negative (Escherichia coli and salmonella typhi) bacteria.

Table 2: Anti-Bacterial Activity of SC-CO_2 Extract

Sr .No	*Type of bacteria*	*MIC of extract (µ g/ml)*	*MBC of standard drugs (µ g/ml)*			
			A	*B*	*C*	*D*
1	E.COLI (MTCC 443)	100	0.05	50	25	10
2	P.AERUGINOSA (MTCC 1688)	125	1	50	25	10
3	S.AUREUS (MTCC 96)	200	0.25	50	50	10
4	S.PYOGENUS(MTCC 442)	100	0.5	50	50	10

A=GENTAMYCIN, B=CHLORAMPHENICOL, C=CIPROFLOXACIN, D=NORFLOXACIN

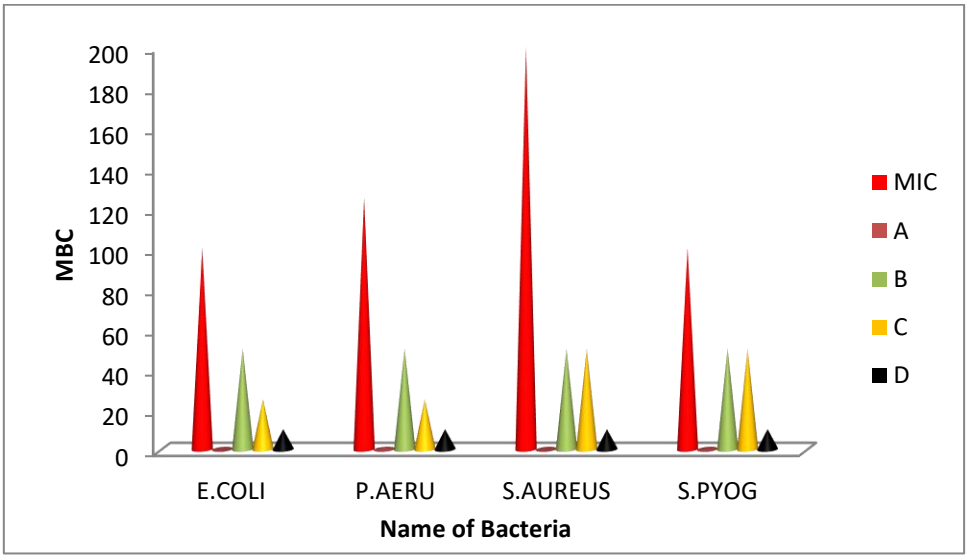

Figure 2: MIC values of bacteria used comparing with MBC of the standard drugs

The SC-CO_2 extract is examined for its anti-fungal activity against three fungus named (C.Albicans,A.Niger and A.Clavatus) and it is detected that the MIC of the extract are (250,1000 and >1000)

µg/ml respectively.

This MIC of the extract are compared with the minimal fungicidal concentration (MFC) of two standard drugs named Nystatin(100,100,100) µg/ml respectively and Greseofulvin (500,100,100) µg/ml respectively .As a result of that it is detected that the SC-CO_2 extract showed a great significant inhibition for C.albicans (250 µg/ml) compared with the standard drug Greseefulvin (500 µg/ml) whereas the inhibitory activity is less compared with other standard drugs. Table (3) represents the MIC values of the extract and MFC values of the standard drugs for every fungi used and figure (3) shows a comparison between them.

Table 3: Anti-Fungal Activity of SC-CO_2 Extract

Sr .No	*Type of fungi*	*MFC of extract (µ g/ml)*	*MFC of standard drugs (µ g/ml)*	
			NYSTATIN	**GRESEOFULVIN**
1	C.ALBICANS(MTCC 227)	250	100	500
2	A.NIGER (MTCC 282)	1000	100	100
3	A.CLAVATUS (MTCC 1323)	>1000	100	100

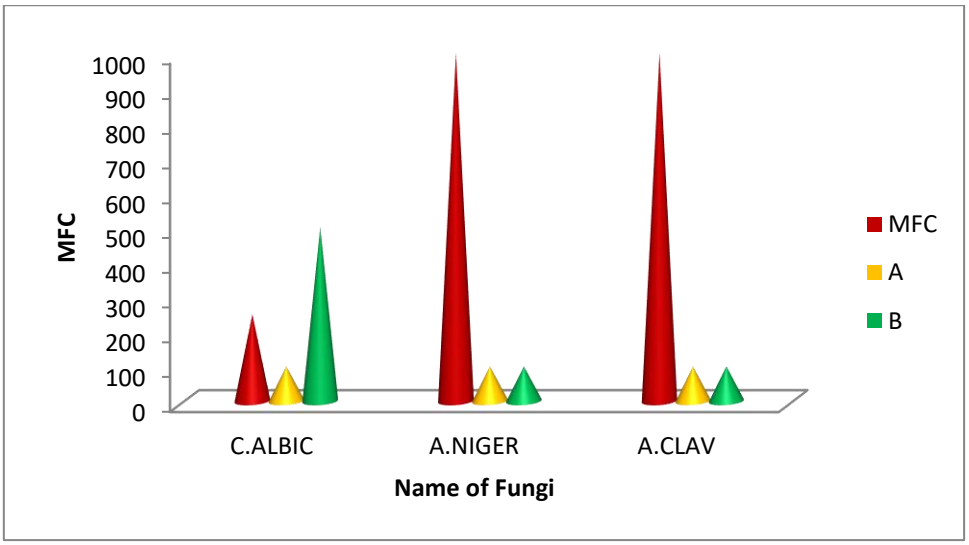

Figure 3. Comparison between MFC values of SC-CO_2 extract and standard drugs

Study of Anti-Tuberculosis and anti-malarial Activity

Table 4: Anti-tuberculosis and Anti-malarial Activities of SC-CO_2 Extract

Sr .No	**Type of bacteria**	**MIC of Extract (µ g/ml)**	**Standard drugs Susceptibility (µ g/ml)**			
			A	**B**	**C**	**D**
1	Anti-tuberculosis	500	0.20	0.25	ND	ND
2	Anti-malaria	1.023	ND	ND	0.020	0.268

A= Isoniazid, B= Rifampicin, C= Chloroquine:IC 50 ,D= Quinine IC 50 , ND: Not Detected

The study of anti-tuberculosis of SC-CO_2 is highlighted .The extract inhibited the growth of mycobacterium tuberculosis .The MIC of the extract is 500 µg/ml whereas the standard drugs susceptibility found to be 0.20 µg/ml for Isoniazid drug and 0.25 µg/ml for Rifampicin drugs so it is revealed that the extract had no significant inhibition comparing with this two standard drugs. In addition to that the anti-malarial activity of SC-CO_2 extract is checked against plasmodium falciparum strain. The MIC of the extract is recorded

as (1.023 µg/ml). The extract possessed moderate inhibition comparing with the two standard drugs used Chloroquine (0.020 µg/ml) and quinine (0.2681 µg/ml). Table (4) showed the MIC of the extract and standard drugs susceptibility for anti-tuberculosis and anti-malarial activities.

CONCLUSION

The qualitative phytochemical screening of the four types of extracts of *chicory* roots revealed that there is some similarity in the presence of some phytocomponents like carbohydrates, alkaloids, phytosterols, fixed oils and fats. However, some extracts show different constituents. The aqueous extract only exhibit the presence of phenols and tannins, whereas the MeOH extract only exhibit the presence of flavonoids. Both aqueous and MeOH extracts showed the presence of amino acids, whereas both SE and MeOH extracts exhibit the presence of cardiac glycosides.

These phytochemical components are responsible for the biological activities which exhibited by the roots of chicory such as anti-bacterial, anti-fungal, anti-oxidant etc. which is reported by previous studies.

The extract of chicory roots which produced by the modern method supercritical fluid extraction has deficiency in the study of its biological activity therefore it is highlighted in this study to explain and detect its several biological activities. Three important activities are examined, anti-Tuberculosis, anti-malaria and ant-microbial. This study showed no significant anti-Tuberculosis activity and moderate anti malaria activity based on the standard drugs which are used for comparing. However, this extract showed great significant anti-fungal activity against C.a itcans in which the inhibition occurred with half MIC needed for standard drug that may be due to the presence of important phytochemical compounds detected in this type of extract such as carbohydrates, alkaloids, phytosterols, fixed oil and fats. Further biological activities are suggested to study and evaluate such as anti-oxidant and anti-cancer activities which expected to exhibit by the extract due to the availability of active phytochemical compounds which will help to reveal the importance of chicory roots in medicinal field and solving the health problems especially that spread in our days.

REFERENCES

[1] Kocsis I., Hagymasi K., Kery A., Szoke E., Blazovics A., Effects of chicory on pancrease status of rats in experimental dyslipidaemia., Acta Biol. Szeged., 2003, 4:143-146.

[2] Ozurk, D., Bal M., Erol A., Sahin M., Ozkan C., Karakas E., Ata M., Karabay P., Determination of nutritive value of wild chicory forage harvest different maturity stage using in vitro and in situ measurements. Pakistan J.of Biol. Science, 2006., 9 (2):253-259.

[3] Jung G., Shaffer J., Varga, Everhart G., J. Agron., 1996, 88,: 104 – 111

[4] Gazzani G, Daglia M, Papetti A, Gregotti C., In vitro and ex vivo anti- and prooxidant components of Cichorium intybus. J.Pharm Biomed Anal., 2000, 23: 127-133

[5] Papetti A, Daglia M, Gazzani G., Anti- and prooxidant activity of water soluble compounds in Cichorium intybus var silvestre (Treviso red chicory). J Pharm Biomed Anal., 2002, 30: 939-945.

[6] Zafar R., Ali S., Anti-hepatotoxic effect of root and root callus extracts of Cichorium intybus L. J Ethnopharmacol., 1998, 63, p. 227.

[7] Ahmed B., Tawfeq A., Howiriny A., Siddiqui B.,Antihepatotoxic activity of seeds of Cichorium intybus.J. Ethnopharmacol., 2003, 87(2-3) : 237-240.

[8] Pushparaj P., Low K., Manikandan J, Tan B., Tan C., Anti-diabetic effects of Cichorium intybus in streptozotocin-induced diabetic rats. J Ethnopharmacol; 2007,111 (2): 430-434.

[9] Petrovic J, Stanojkovic A, Comic L, Curcic S., Antibacterial activity of Cichorium intybus. Fitoterapia, 2004, 75: 737-739

[10] Ilaiyaraja N, Khanum F., Evaluation of Antioxidant and Toxicological properties of Chicory leaves. International Journal of Pharmaceutical & Biological Archives, 2010, 1(2): 155 – 163

[11] Tiwari S., I Jour.of Nat. Products, 2008,vol.1: 27-35.

[12] Y. Ge, Y. Ni, H. Yan, Y. Chen, T. Cai, Journal of Food Science 2002,67 : 239.

[13] Ola Basa'ar, Samreen Fatema, Ali Alrabie, Mazahar Farooqui., Supercritical Fluid Extraction of Cichorium intybus (L) and it's Characterization ., Int.J.chem. Pharm.Sci, 2016,9(4) : 2936-2944

[14] Hongzhi Cao, Joel Hwang and Xi Chen., Carbohydrate-containing natural products in medicinal chemistry, Opportunity,Challenge and Scope of Natural Products in Medicinal Chemistry, 2011, 37/661 (2),411-431,978-81-308-0448-4.

[15] Nobori, T., Miurak, K., Wu, D.J., Takabayashik, L.A, Carson,D.A.,Deletion of cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature, 1994, 46, 753-756.
[16] RNS Yadav and Munin Agarwala, Phytochemical analysis of some medicinal plants. J.Phytology, 2011, 3(12),10-14, 2075-6240.
[17] Antherden, L.M., Textbook Of Pharmaceutical Chemistry ,8th edn., Oxford University Press, London, 1969,: 813-814.
[18] Harborne, J.B. Phytochemicals Methods . Chapman and Hall Ltd., London, 1973, 49-188.
[19] Stray, F. The Natural Guide to Medicinal herbs And Plants . Tiger Books International, London, 1998. 12-16.
[20] Okwu, D.E., Okwu, M.E., Chemical composition of Spondias mombin linn. plant parts. J. Sustain. Agric. Environ., 2004, 6(2):140-147.
[21] R.E. Ostlund Jr, Annual Review of Nutrition.Phytosterols in human nutrition,2002 .
[22] Bano Rafia, Phytosterols in Human Nutrition. IJSRR. 2013, 2(2), 01- 10, 2279–0543.
[23] Anderson JW, Allgood LD, Lawrence A, et al., Cholesterol-lowering effects of psyllium intake adjunctive to diet therapy in men and women with hypercholesterolemia: meta-analysis of 8 controlled trials . Am J Clin Nutr.; 2000, 71:472-479.
[24] T.A., Woyengo et al , Anticancer effects of phytosterols. Eur.J. Clin. Nutrition,2009.
[25] Wasserman SI., Approach to the person with allergic or immunologic disease. In: Goldman L, Schafer AI, eds. Cecil Medicine. 24th ed.Philadelphia, Pa: Saunders Elsevier, 2011,chap 257.
[26] Antonio M. Rabasco Alvarez and María Luisa González Rodríguez, Lipids in pharmaceutical and cosmetic preparations, 2000 ,51, 1-2: 74-96.
[27] Ali, S.S., Kasoju, N., Luthra, A., Singh, A., Sharanabasava, H.,Sahuand, A., Bora, U. Indian medicinal herbs as source of antioxidants. Food Res. Int., 2008, 41: 1-15.
[28] Marjorie, C., Plant products as antimicrobial agents .Clincal Microbiol. Rev., 1996, 12, 564-582.
[29] Swati M. Devare1, Gayatri S. Kamble1, Rasika C. Torane1, Asha A.Kale1, Nirmala R. Deshpande1 and Jyoti P. Salvekar, J. Chem. Pharm. Res., 2011, 3(3):192-195, 0975-7384.
[30] Nyarko, A.A., Addy, M.E. Effects of aqueous extract of Adenia cissampeloides on blood pressure and serum analyte of hypertensive patients. Phytotherapy Res., 1990, 4(1), 25-28.
[31] Schoner, W., Scheiner-Bobis, G., Am J Cardiovasc Drug, 2007, 7,:173.
[32] Newman, R.A., Yang, P.Y., Pawlus, A.D., Block, K.I. Mol., Interv., 2008, 8: 36.
[33] S. Nandagopal and B.D. Ranjitha Kumari , Phytochemical and Antibacterial Studies of Chicory(Cichorium intybus L.) - A Multipurpose Medicinal Plant, Adv.Biolog.Res., 2007, 1 (1-2): 17-21, 1992-0067.
[34] Parinita Sarma , Ramen Kalita and Dibyendu Shil, Journal of Drug Discovery and Theraputics, 2014, 2(18):2320-4230.
[35] Saxena Mamta and Saxena Jyoti, Phytochemical Screening Of Acorus Calamus and Lantana Camera. Int.Res.J.Pharm., 2012,3(5):2230-8407.