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Original Article

GC-MS Analysis, Antioxidant, Antimicrobial and Anticancer Activities of Extracts from *Ficus sycomorus* Fruits and Leaves

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Abstract

Higher plants have been utilized worldwide as characteristic drug a long time to cure human diseases. About 80% of individuals globally use plants as safe sources of medication to cure human diseases through completely different medicine system. One of the available indigenous medicinal plants, *Ficus sycomorus* belongs to the Moraceae family. The plant contains totally different teams of biologically active compounds that square measure chargeable for the biological activity. Ethanolic and ethyl acetate extracts of leaves of *Ficus sycomorus* contain higher concentrations of total phenols, flavonoids, tannins, alkaloids and steroids than the fruit extracts. Ethanolic extract in both fruits and leaves gave higher concentrations of phytochemical compounds than the ethyl acetate extracts. Therefore, fruit and leaves extract have antioxidant and antimicrobial activity against gram positive, negative bacteria and fungus. Also, the percentage of Liver cell line (HepG2), Colorectal adenocarcinoma (Caco-2) and Breast cell line (MCF-7) viability was decreased with increasing the concentrations of the ethanolic extract of fruits and leaves of *Ficus sycomorus*. The high concentrations of ethanolic extract of fruits caused high reduction in the viability of cancer cells, especially in Colorectal adenocarcinoma (Caco-2) cell line. In addition, phytochemical compound screened by GC-MS method. In GC-MS analysis, 12 bioactive phytochemical compounds were identified in fruits and 29 bioactive compounds were detected in leaves extract. These totally different active phytochemicals are found to possess a good vary of activities, which can facilitate within the protection against incurable diseases.

Keywords: antibacterial; anticancer activity; DPPH; fatty acids; flavonoids; phenols; steroids

Introduction

Ficus sycomorus is a short tree that belongs to the Moraceae family. It is native to Africa. Its fruit is called figs, which have been used as food and medicine. Locally, it is called 'subula', and it is widely distributed in the Mediterranean basin of Egypt and called 'sycamore' or 'gimmeiz'. Among the important medicinal plants are species that belong to Moraceae, often called the mulberry family or fig family. This is a family of flowering plants comprising approximately 40 genera and over 1000 species. Most are widespread in tropical and subtropical regions (Van *et al.*, 2007). The height of *F. sycomorus* is approximately 20 m and 6 m wide. The fruit is large, approximately 2-3 cm in diameter, maturing from buff-green to yellow or red. The bark is a green or yellow to orange colour. The leaves are heart-shaped with a spherical apex. They are 14 cm tall and 10 cm broad. Also, their margin is entire. The flower is spherical, greenish, and unisexual. It contains latex, like all other figs (Romeh, 2013). The fruits of *F. sycomorus* are an important herbal medicine and food that is used to treat fungal infections, jaundice and dysentery (Hassan *et al.*, 2007). In addition, it is used for the treatment of cough, diarrhea, skin infection, stomach disorders, liver disease, epilepsy, tuberculosis, lactation disorders, helminthiasis, infertility and sterility (Sandabe *et al.*, 2006). The extracts of fruits of *F. sycomorus* are also used for the treatment of various diseases, such as cough, diarrhea, stomach disorder, skin infections, epilepsy, liver disease, tuberculosis, lactation disorder, infertility and sterility and helminthiasis (Sandabe *et al.*, 2007; Bello *et al.*, 2013). The target plant contains several bioactive chemical constituents. The most important bioactive constituents of

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this plant are alkaloids, tannins, flavonoids and phenolic compounds (Zaku *et al.,* 2009). *Ficus Sycomorus* also has been shown to possess antioxidant, antibacterial, hypolipidemic, and hypoglycemic activities (Lansky *et al.,* 2008; Ao *et al.,* 2008; Abdel-Hameed, 2009). Phytochemical and toxicity evaluation on the stem bark of *F. sycomorus* L was carried out by Ibrahim *et al.* (2006) on mice with LD50 value of 471.1 mg/kg. Its chemical constituents were found to include tannins, resins, steroid glycoside, reducing sugars and saponins. The extract is said to be moderately toxic to mice and therefore can be safely used ethno-medically at lower doses (Bello *et al.,* 2015).

The aim of this work was to study the chemical composition of leaves and fruits of *Ficus sycomorus* L. and to study their effects as antioxidant, antimicrobial and anticancer activity. In addition, to determine the active compounds separated by GC-mass.

Materials and Methods

Plant material

Leaves and fruits of *Ficus sycomorus* L. (Moraceae) were collected from Faculty of Agriculture, Cairo University, Giza, Egypt. *Ficus sycomorus* was botanically characterized by Prof. Dr. Mohamed Osama from Plant taxonomy Department, Faculty of Agriculture, Cairo Univeristy, Egypt.

Microbial strain

Table 1 illustrated the microorganisms which were used in this study and were obtained from the American Type Culture Collection (ATCC) as well as the culture collection of the Microbiology Lab, Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University.

Extraction method

The fruits and leaves were cleaned and washed thoroughly under tap water, and then freeze-dried and grinded into fine powder using an electric blender. The powder was dried in an oven at 40 °C for 24 h. The fine powder sample (500mg) was extracted in 10 ml ethanol or distilled H_2O for 24 h using a shaker, then the extract was filtered and the samples were stored at 4 °C till use (Sumathy and Sumathy, 2011). All analysis was done in the labs of Cairo University, Research Park (CURP), Cairo University, Faculty of Agriculture, Cairo, Egypt.

Phytochemical composition of Ficus sycomorus

The total phenolic content was estimated by Folin Ciocalteu method as described by Singleton and Rossi (1965). The absorbance was measured at 765 nm using a spectrophotometer Thermo Scientific HERYIOS. Total phenolic content was expressed as mg gallic acid equivalent (GAE) per g dry weight. The flavonoids content was determined by aluminium trichloride method as described by Zhishen *et al.* (1999). The absorbance was determined at 510 nm using a spectrophotometer. Results expressed as mg Quercetin /g dry weight. Tannin content in fruits and leaves of *Ficus sycomorus* was determined by using Folin-Denis reagent as described by Saxena *et al.* (2013). The absorbance was scan at 700 nm using spectrophotometer. Results expressed as mg Tannic acid /g dry weight. Alkaloids were measured according to the method described by Harborne (1998). The absorbance was taken at 565 nm. The alkaloid concentration was calculated from the calibration curve of atropine used as standard and results expressed as g/100 g equivalent of atropine. The total anthocyanin contents were determined by the pH differential method (Lee *et al.*, 2005) using a spectrophotometer (Thermo Scientific HERYIOS). The absorbance of the fruit extract was determined at 515 and 700 nm at pH 1.0 and 4.5 buffers, respectively, using A = (A515 - A700) pH 1.0 - (A515 - A700) pH 4.5 with a molar extinction coefficient of 26,900. The results were expressed as mg of cyanidin-3-glucoside equivalent per 100 grams of fresh weight (g cy-3-glu kg^{-1} FW).

Lipid extraction

The fruit and leaves of *Ficus sycomorus* oil content were determined using the Soxhlet extraction according to the official method (AOAC, 2000). Fifty g of dried fruits and leaves were ground and then extracted with petroleum ether in a Soxhlet apparatus for 6 h. After extraction, the samples were ground again, but more finely, and extracted for 6 h (second extraction). Petroleum ether was vaporized under reduced pressure employing a rotavapor. Lipid content was expressed as g/100 g of fresh weight.

Separation of fatty acids and unsaponifiables from lipid samples

Lipid material was saponified with methanolic KOH (40%, w/v) for 24 h at room temperature according to Ahmed *et al.* (1986). The unsaponifiables were extracted three times with ether. The aqueous layer was acidified with HCl (1:1, v/v) and the liberated fatty acids were extracted three times with ether. The combined extracts of unsaponifiables and fatty acids were washed many times with distilled water and then dried over anhydrous sodium sulfate. The standard and the sample fatty acids were converted to methyl esters using ethereal solution of diazomethane according to Vogel (1975).

Table1. Microbial strains used to test the antimicrobial activities of *Ficus sycamorus* fruit and leaves extract

Microbial group	Indicator strain	Positive control	Cultivation conditions
Gram positive bacteria	*Staphylococcus aureus* (ATCC 25923)	Kanamycin	Muller-Hinton broth, 37 °C/ 24 h
	Bacillus cereus (ATCC 33018)		Muller-Hinton broth, 30 °C / 24 h
Gram negative bacteria	*Escherichia coli* (ATCC 8739)	Polymyxin	Muller-Hinton broth, 37 °C / 24 h
	Salmonella typhimureum (ATCC 14028)		Muller-Hinton broth, 37 °C / 24 h
Fungus	*Aspergillus niger* (nrrl 326)	Nystatin	Sabouraud dextrose broth, 25 °C / 3days
	Candida albicans ATCC 10231		Sabouraud dextrose broth, 25 °C / 24 h

Determination of fatty acid composition by (GC-MS)

The methyl esters of fatty acid were measured by GC–MS using Trace GC Model 2000 series created by Thermo equipped with Selective Detector Mass Spectroscopy Model SSQ 7000 created by Finnigan. This equipment was interfaced via HP chemstation version A 02.12 software (Hewelwett-Packard, Avondale, PA). The gas chromatography was equipped with DB-23 (J & W 122-2362) 25μ capillary column, 60 m × 0.25 mm ID, and 0.15 μm. The operational conditions for gas chromatography were as follows: injector temperature 250 °C, carrier gas: helium at 30 cm/sec, measured at 150 °C, oven temperature 50 °C for 4 min, 150 °C for 4 min and held at 250 °C until the chromatogram was completed. The detector temperature was 280 °C. Mass spectroscopy operational parameters were electron ionization at 70 ev, accelerating voltage 10 kV and scan M/Z range from 50 to 500, National Institute of Standards and Technology (NIST) library according to Jiang *et al.* (2006).

Determination of sterols profile by GC-MS

The unsaponifiable fractions were finally collected in ether and brought to condition beneath vacuum. The residue was analyzed using the gas chromatograph HP 5890 (Hewlett Packard) equipped with the MS detector (MSD 5970), EI, 70 ev and fitted with a capillary column DB-1701 (12 m × 0.18 mm × 0.4 mm; J&W Scientific). The column temperature was programmed from 260 to 300 °C whereas injection temperature was set at 280 °C. Helium was the carrier gas at a rate of flow of 0.7 cm^3/min. Identification of peaks was supported the retention time of standard substances and MS spectra. Analyses were run in triplicate. Calculations of percent composition of demethylsterol fractions were based on the peak area.

Extraction of phenolic and flavonoid compounds

Dry sample (0.2 g) extracted with 20 ml ethanol 80%, soak in brawn bottle for 24 hr at room temperature, centrifuged for 5 min, volume adjusted to 25 ml by ethanol 80%, filtered through Whatman filter paper, 10 ml of the solution evaporated to dryness then dissolved in 5 ml HPLC grade methanol 50%, filtered through PTFE filter with pore size 0.2 μm.

Instrument condition for phenolic compounds

Agilent 1260 infinity HPLC Series (Agilent, USA), equipped with Quaternary pump, a Zorbax Eclipse plusC18 column 100 mm x 4.6 mm i.d., (Agilent technologies, USA), operated at 30 °C. The separation is achieved using a ternary linear elution gradient with (A) HPLC grade water 0.2% H_3PO_4 (v/v), (B) methanol and (C) acetonitrile. The injected volume was 20 μl. Detection: VWD detector set at 284 nm.

Instrument condition for flavonoids

HPLC, Smart line, Knauer, Germany., equipped with binary pump, a Zorbax Eclipse plusC18 column 150 mm x 4.6 mm i.d. (Agilent Technologies, USA), operated at 35 °C. Eluent: methanol: H_2O with 0.5% H_3PO_4, 50:50 with flow rate 0.7 ml/min, the injected volume was 20 μl. Detection: UV detector set at 273 nm and data integration by claritychrom@ software. This method was the modified of methods Goupy *et al.* (1999) and Mattila *et al.* (2000) for fractionate the polyphenols and flavonoids, respectively.

DPPH free radical scavenging activity (RSA)

The antioxidant activity of fruits and leaves of *Ficus sycomorus* extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable DPPH method as modified by Hae-Ryong *et al.* (2006). The reaction mixture containing 1 ml of the extract at different concentrations (40, 80, 120, 150 μg/ml) and 1ml of DPPH (0.2mM) was vigorously shaken and incubated in darkness at room temperature for 30 minutes. The absorbance was browse at 517nm exploitation UV-visible photometer. Radical scavenging activity was expressed as percent of inhibition and was calculated exploitation the subsequent formula:

%DPPH = [Absorbance of Control – Absorbance of Sample / Absorbance of Control] × 100

Determination of reducing power

The ability of the tested extracts to reduce Fe^{3+} was assayed by the method of Chou *et al.* (2009). The absorbance was measured at 700 nm. The results were expressed as μg of gallic acid equivalent per 100 g DW.

Antibacterial activity

Agar disc diffusion method was used to evaluate the antibacterial activity of fruit and leaves of *Ficus sycomorus* as describe by Bauer *et al.* (1966). The strains were grownup on Mueller-Hinton agar slants at 37 °C for 24 h and checked for purity. After the incubation, the cells were washed off the surface of agar and suspended in sterile physiological solution. The number of cells in 1 ml of suspension for inoculation measured by McFarland nefelometer was 5 × 107 CFU/ml. 1 ml of those suspensions was homogenized with 9 ml of liquified (45 °C) Mueller-Hinton agar and poured into Petri dishes. On the surface of the agar, 5 mm diameter paper discs (HiMedia®, Mumbai, India) were applied and impregnated with 15 μl of samples. The plates were incubated at the optimum temperature for every indicator strain (Table 1) and tested when 24, 48 and 72 h. Growth inhibition was scored positive within the presence of a detectable clear zone (ZI) round the disc and expressed in mm. Experiments were carried out in triplicates and the inhibition zone was recorded as the average of the replicates ± SD.

In vitro cytotoxicity assay

Liver cell line (HepG2), colorectal adenocarcinoma (Caco-2) and Breast cell line (MCF-7) were purchased from CURP, faculty of Agriculture at Cairo University (Egypt). Cells were maintained in (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 μg/ml streptomycin and 100 units/ml penicillin g potassium, in a humidified 90% and 5% (V/V) CO_2 atmosphere at 37 °C. The cytotoxicity of ethanolic extracts was tested by the neutral red (NR) assay as previously described by Repetto *et al.* (2008). Exponentially growing cells were collected using 0.25% Trypsin-EDTA and seeded in 96- well plates at

20000 cells/well. After incubation (overnight), extracts were added in various concentrations (10, 50, 100, 200, 400, and 800 µg/ml); 4 wells for each concentration. After treatment with extracts for 24 h, media were removed and cells were exposed to neutral red solution for 4 hours at 37 °C. Destin solution was used to dissolve the NR stained cells and color intensity was measured at 540 nm microplate reader (Biotek, ELX808).

The fruits and leaves of Ficus sycomorus extraction for GC/MS analysis

The fruits and leaves were cleaned, shade dried and pulverized to a powder in a mechanical grinder. Required amount of powder was weighed and transferred to dramatist flask and treated with methanol till the powder is absolutely immersed. The flask was jolted each hour for the primary 6 hours and so it had been unbroken aside and once more jolted after 24 hours. This method was recurrent for 3 days and so the extract was filtered. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The final residue obtained was then subjected to GC-MS analysis.

GC-MS analysis

GC-MS analysis of these extracts was performed using an Agilent 7000 Series Triple, Quad Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS/) equipped With a Elite-5MS (5% diphenyl/ 95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25µm df)) For GC-MS detection an electron Ionization system with ionizing energy of 70ev was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate 1ml/min and injection volume of 2µl was employed (split ratio of 10:1); injector temperature 250 °C; ion-source temperature 200 °C. The oven temperature programmed from 110 °C (iso thermal for 2 min) With an increase of 10 °C/min to 200 °C, then 5 °C/ min to 280 °C, ending with a 9 min iso thermal at 280 °C, mass spectra were taken at 70ev: a scan interval of 0.5 second and fragments from 45 to 450Da, total GC Running time was 36 minutes. The relative % amount of each component was calculated by comparison its average Peak area to the total areas. Software adopted to handle mass spectra and chromatograms was Turbomass.

Interpretation of mass spectrum GC-MS was conducted using the database of Nationl Institue of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Bagavathi and Ramasamy, 2012).

Statistical analysis

All results were expressed as mean values ± standard deviation. Comparisons were performed by analysis of variance (ANOVA). Statistical analyses were run using SAS software.

Results and Discussion

Phytochemical compounds of fruits and leaves of Ficus sycomorus

The phytochemical compounds of the two extracts of fruits and leaves of *Ficus sycomorus* are presented in Table 2. The ethanolic and ethyl acetate extracts of leaves of *Ficus sycomorus* have higher concentrations of total phenols, flavonoids, tannins, alkaloids and anthocyanin than the fruit extracts. The ethanolic extract in both fruits and leaves of *Ficus sycomorus* gave higher concentrations of phytochemical compounds than the ethyl acetate extract. These results are in accordance with Mudi *et al.* (2015) who found that the phytochemical analysis of of *Ficus sycomorus* revealed the presence of alkaloids, tannins, saponins, flavanoids and steroids in both the aqueous extracts of the leaves and the fruits. These classes of compounds are known to be biologically active and are associated with the antimicrobial activities of *Ficus sycomorus* (Kesba and El-Beltagi, 2012; Mohamed *et al.,* 2013; El-Beltagi *et al.,* 2018). Alkaloids have been associated with medicinal applications in plants, among which is their toxicity against cells of foreign organisms. These bioactivities have been widely studied for their potential use in the inhibitory activities of human cancer cell lines (Nobori *et al.,* 1994; Akinpelu *et al.,* 2008). Alkaloids inhibit certain mammalian enzymatic activities like those of phosphodiesterase, prolonging the action of CAMP. They additionally have an effect on glucagons and thyroid stimulating hormones, while some forms of alkaloids which extracted from *Rhazya stricta* have been reported to be carcinogenic (Soonham, 2015). However, some alkaloids are used either as an analgesic, antispasmodic or bactericidal agents (Tim-Cushnie, 2014). Plant phenolic compounds, especially flavonoids are currently of growing interest owing to their supposed properties in promoting health (antioxidants) and antimicrobial activity (Rauha *et al*., 2000; Abd El- Rahman *et al*., 2012). Flavonoids also exhibit a wide range of biological activities such as antimicrobial, anti-inflammatory, analgesic and cystostatic, hypoglycemic and antioxidant properties (Scalbert, 1991; Hodek *et al.,* 2002; Abdel-Rahim and El-Beltagi, 2010). The broad therapeutic effects of flavonoids will be mostly attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages (Mark, 1998; Shallan *et al*., 2010). Tannins are reported to inhibit the growth of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them; they form irreversible complexes with proline rich proteins, resulting in the inhibition of the cell protein synthesis. They have important roles such as stable and potent antioxidants (Ogunleye and Ibitoye, 2003; Kobeasy *et al*., 2011). Plant tannin has been recognized for their pharmacological properties and is thought to form trees and shrubs a difficult meal for several caterpillars (Aiyelaagbe and Osamudiamen, 2009).

Fatty acid and sterols compounds of fruits and leaves of Ficus sycomorus

The ethanolic extract of fruits and leaves of *Ficus sycomorus* contains 10 fatty acid compounds, 7 compounds saturated fatty acids and 3 compounds unsaturated fatty acids (Table 3). The main constituent is C18:2 linoleic acid followed by C18:1 oleic acid and C18:3 linolenic acid. Leaves extract contains high amount of saturated fatty acids (27.28%) than fruit extract which contain 26.58%. On the other hand, fruit extract contains high amount of

unsaturated fatty acids (73.42) than leaves extract which contain (72.72%). These results are in accordance with Ivanov *et al*. (2018) who found that the highest value of polyunsaturated fatty acid α-linolenic acid C18:3 and linoleic acid C18:2 were established in all extracts of fig (*Ficus carica* L.) leaves. It is well known that polyunsaturated fatty acids can influence some physical properties of the cellular membranes such as fluidity and permeability (Ward and Singh, 2005). Moreover, the benefits of polyunsaturated fatty acids in some diseases, such as cardiovascular diseases and autoimmune disorders, have been reported (Reiffel and McDonald, 2006; Afify *et al.,* 2018). Results clearly showed that the fruits and leaves extract of *Ficus sycomorus* contained more unsaturated fatty acids as α-linolenic acid and linoleic acid. These natural components have been used as co-emulsifiers, emollients and thickeners in cosmetics and food industry (Pejin *et al*., 2014). Also, oleic acid ester is known to have potent antibacterial and antifungal (Seidel and Taylor, 2004). Oleic acid in concentrations as low as 0.7% (v/v) has been found to be fungistatic against a wide of molds and yeasts (Sheba *et al.,* 1999).

Phytosterols inhibit the absorption of intestinal and endogenous biliary cholesterol. Plants contain free and esterified phytosterols which will be acylated with β-sitosterol, campisterol and stigmaserol. These phytochemical compounds play important role in cellular processes such as regulation of membrane fluidity, adaptation of membranes to temperature, participation in cellular differentiation and proliferation (MacKay and Jones, 2011).

The results in Table 3 showed that the ethanolic extract of fruits and leaves of *Ficus sycomorus* contain five compounds of sterols sperated by HPLC. The most constituent is β-Sitosterol followed by Stigmasterol and Campesterol. Leaves extract contain high amounts of β-Sitosterol and Campesterol than fruits extact. These results are in accordance with Ivanov *et al*. (2018), who found that β-sitosterol given the most phytosterol within the fig leaves. This compound could be a non-competitive inhibitor of 5-α-reductase and it possesses proved anti-inflammatory drug impact thanks to inhibition of 5-lipoxygenase pathways of arachidonic acid (Cabeza *et al*., 2003; Prieto *et al*., 2006).

Plant steroids are renowned to be necessary for their cardiotonic, insecticidal and anti-microbial properties. They are conjointly utilized in nutrition, herbal medicines, cosmetics and they are habitually utilized in drugs due to their profound biological activities (Denwick, 2002). Steroids present in plants are cardiotonic in nature and are reported to have antidiabetic and anti-fungal properties (Kamel, 1991). They are stocked in plant cells as inactive precursors but are readily converted into biological active antibiotics by enzymes in response to microorganism attack.

Table 2. Quantitative phytochemical analysis of *Ficus sycamorus* fruit and leaves extracts

Constituents	Fruits extracts		Leaves extracts	
	Ethanolic	Ethyl acetate	Ethanolic	Ethyl acetate
Total phenolic (mg Gallic acid /g DW)	51.88±2.04[a]	33.55±1.38[b]	63.54±0.53[a]	44.22±0.94[b]
Total flavonoid (mg Quercetin /g DW)	8.57±1.01[a]	2.48±0.16[b]	12.58±0.01[a]	5.58±0.06[b]
Total tannin (mg Tannic acid /g DW)	7.11±0.22[a]	4.92±0.28[b]	11.11±0.23[a]	6.14±0.09[b]
Total alkaloid (g/100g DW)	4.03±0.43[a]	3.07±0.13[b]	8.42±0.34[a]	5.57±0.18[b]
Total anthocyanin (mg cy-3-glu /100g FW)	113.44±0.84[a]	86.17±0.55[b]	142.51±0.43[a]	113.66±0.36[b]

Values are mean ± SD of three replicate analyses

Table 3. Fatty acids and sterols compositions percent of *Ficus sycamorus* oils

Lipid Composition	Constituents	Ethanolic extract of fruits	Ethanolic extract of leaves
Fatty acids	C12:0 Lauric acid[a]	0.31	0.22
	C14:0 Myristic acid[a]	3.61	3.33
	C16:0 Palmitic acid[a]	10.12	10.59
	C18:0 Stearic acid [a]	11.50	12.46
	C18:1 Oleic acid	24.06	27.32
	C18:2 Linoleic acid	30.03	29.82
	C18:3 Linolenic acid	19.34	15.58
	C20:0 Arachidic acid[a]	0.27	0.062
	C22:0 Behenic acid[a]	0.19	0.092
	C24:0 Lignoceric acid[a]	0.59	0.53
	[a]SFA	26.58	27.28
	[b]USFA	73.42	72.72
	USFA/SFA	2.76	2.67
Sterols	Campesterol	10.15	11.88
	Stigmasterol	15.62	10.39
	Lanosterol	2.73	1.86
	β-Sitosterol	70.66	75.61
	Δ^5-Avenasterol	0.84	0.26

[a]Total saturated fatty acids. [b]Unsaturated fatty acids

Phenolic and flavonoid compounds of fruits and leaves of Ficus sycomorus

Medicinal plants have a large sort of phenolic compounds, like flavonoids that act potentially as antioxidants, scavenging free radicals, reactive oxygen species and inhibit lipid peroxidation (Kumawat *et al.*, 2012).

The ethanolic extract of fruits and leaves of *Ficus sycomorus* contains 18 phenolic compounds (Table 4). The most constituent in fruits extract is Benzoic acid followed by Vanillic acid and Caffeine. Additionally, the most constituent in leaves extract is Ellagic acid followed by Rutin and Chlorgenic acid. Also, Pyrogallol, *p*-Hydroxy benzoic acid and Chlorgenic acid found only in leaves extract. While, Quinol and o- Coumaric acid found only in fruits extract. Similar results are reported by El-Sayed *et al.* (2010) who found that Gallic acid, quercetin, rutin, β-Sitosterol glucopyranoside, isoquercitrin and quercetin galactopyranosyl (1→6) glucopyranoside are the compounds that isolated from the leaves of *Ficus sycomorus*.

In addition, the ethanolic extract of fruits and leaves of *Ficus sycomorus* contains 6 flavonoids compounds like Myricetin, Cinnamic acid, Quercitin, Rosmarinic acid, Neringenin and kaempferol. While, Quercitin found only in fruit extract. Blasiak *et al.* (2002) found that Quercetin, beside the vitamin C, might play a crucial role within the protection of DNA molecules from harm and therefore stop the appearance of mutations and cancer. Quercitrin and Rutin is a quercetin 3-glucosid with significant antioxidative and antibacterial effects (Arima *et al.,* 2002).

Fruit extract contains high amounts of Kaempferol than leaves extract. Also, high amounts of Myricetin and Neringenin found in leaves extract than fruit extract. Rauha *et al.* (2000) found that kaempferol and its derivatives have antibacterial activity.

Antioxidant activity of fruits and leaves of Ficus sycomorus

Antioxidants are substances that defend living cells from the harm caused by unstable molecules known as free radicals. Antioxidants are renowned to act and stabilize free radicals, thereby preventing harm. The free radical damage might result in the development of cancer (Prior *et al.*, 2005). Antioxidant molecules are capable of retarding or preventing the oxidation of other molecules. Oxidation refers to the chemical reaction that transfers electrons from one substance to another. Oxidation reactions produce free radicals that begin chain reactions that harm the cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibiting other oxidation reactions. Examples of some antioxidants are Beta-carotene, lycopene, vitamins A, C and E (Lopez *et al.*, 2007).

The ethanolic extract of each fruits and leaves of *Ficus sycomorus* gave the high antioxidant activity as compared with ethyl acetate extract and also the leaves extract which gave the hight pronounced activity than fruit extract. The scavenging of DPPH radicals inflated with increasing extract concentration from 40, 80, 120 and 150 µg /ml (Table 5). The IC_{50} value of ethanolic and ethyl acetate extracts of leaves was below than fruits extract. IC_{50} values indicate the concentration of the test sample needed to inhibit 50% of the free radicals. The IC_{50} value could be a parameter widely applied to measure the free radical scavenging activity (Cuvelier *et al.,* 1992); a smaller IC_{50} value corresponds to a higher antioxidant activity.

The reducing power capacity reflects the presence of an antioxidant for the reduction of ferricyanide ions [Fe (CN) 6]$^{3-}$ to ferrocyanide ions [Fe (CN) 6]$^{4-}$. This reducing property is mostly related to the presence of a reducer exercising an antioxidant action by breaking the free radical chains; yielding a hydrogen atom (Gordon, 1990). The ethanolic extract of each fruits and leaves of *Ficus sycomorus* gave higher reducing power activity than ethyl acetate extract and the leaves extract gave higher reducing power activity than fruit extract (Table 5).

Antioxidant activities of stem bark extracts of *F. sycomorus* using DPPH radical scavenging activity, hydrogen peroxide scavenging activity and ferric reducing antioxidant power showed that the extracts significantly ($p < 0.05$) exhibited robust antioxidant activity compared to the standard (L-Ascorbic Acid) at the concentrations used (Daniel and Dluya, 2016). The antioxidant activity of the extracts may be associated with the high content of phenolics, tannins and flavonoids. The effects of the extracts could be due to the biological systems that are connected to their ability to transfer electrons to free radicals, chelate metals, activate antioxidant enzymes, reducing radicals of alpha-tocopherol or to inhibit oxidases (Bruneton, 2009).

The free radicals that are concerned within the process of lipid peroxidation are considered to play a serious role in varied chronic pathologies such as cancer and cardiovascular diseases among others. Therefore, the ability of the plant extracts as free radical scavenger disclosed that these extracts may use as new of natural antioxidants and prevent the reactive radical species from reacting biomolecules such as lipoproteins, polyunsaturated fatty acid (PUFA), DNA, amino acids, proteins and sugars in susceptible biological and food systems (Chew *et al.,* 2008; El-Beltagi, 2011).

Antimicrobial activity of fruits and leaves of Ficus sycomorus

The antimicrobial activity of fruits and leaves of *Ficus sycomorus* extracts varied according to the species of bacteria and fungi tested and the solvents used (Table 6). The data indicated that the ethanolic extract of fruits and leaves of *Ficus sycomorus* exhibited the highest antimicrobial activity against the investigated food pathogens as compared with the ethyl acetate extract. In addition, leaves extract produced potent antimicrobial activity as compared to fruit extract.

All extracts of fruits and leaves of *Ficus sycomorus* showed higher antibacterial activity (the diameter of zone of inhibition increased) against gram negative bacteria (*Escherichia coli and Salmonella typhimureum*) than gram positive bacteria (*Staphylococcus aureus and Bacillus cereus*). Also, ethanolic and ethyl acetate extract gave higher antifungal activity against *Candida albicans* than *Aspergillus niger*. Similar results are reported by Manimozhi *et al.* (2012) who found that the acetone, methanol and ethyl acetate of *Ficus* spp. showed good antibacterial activity against *P. aeruginosa*, *E. coli*, *P. vulgaris*, *B. subtilis*, and *S. aureus* pathogens.Also, Al-matani *et al.* (2015) reported that the antimicrobial activity of the crude fruit extracts of

Ficus sycomorus showed strong activity against four Gram-positive and Gram-negative bacterial strains, *S. aureus*, *E. coli*, *Haemophilus influenza* and *Proteus spp*. In addition, the fruit extract of *Ficus sycomorus* showed area of inhibition in the test organisms; *E. coli*, *K. pneumoniae, S. aureus, P. aeruginosa* and *P. vulgaris* (Braide *el al.,* 2018).

The antibacterial activity exhibited could be due to the presence of phytochemicals (flavonoids, saponins, terpendois, phenol and tannins) and the occurrence of phenolic compounds in the extract (Ramde-Tiendrebeogo *et al*., 2012).

Anticancer activity of fruits and leaves of Ficus sycomorus

Data in Table 7 showed the cytotoxic activity of *Ficus sycomorus* as an anticancer agent. The percentage of Liver cell line (HepG2), Colorectal adenocarcinoma (Caco-2) and Breast cell line (MCF-7) viability was decreased with increasing the concentrations of the ethanolic extract of fruits and leaves of *Ficus sycomorus* (500, 1000, 1500, 2000, 3000 µg/ml). The most pronounced reduction in the viability of cancer cells was detected after treatment with fruit extract than leaves extract. The high concentrations of ethanolic extract of fruits caused the high reduction in the viability of cancer cells, especially in colorectal adenocarcinoma (Caco-2) cell line. Cancers commonly related to enhanced risk of death and also the noxious facet effects caused by the modern medicine. These results are in accordance with Mousa *et al.* (1994) who showed that the fruit extracts of *F. benjamina*, *F. bengalensis, F. religiosa* and *Ficus sycomorus* exhibited antitumor activity in the potato disc bioassay. Also, the natural and compounds synthesised from *Ficus carica* showed *in vitro* inhibitory effects on proliferation of various cancer cell lines (Joseph and Raj, 2011).

The anticancer effect of ethanolic extract may be due to the presence of poly phenols that play an important role in antioxidant activity (Wu *et al.,* 2004) and have evidently shown antiproliferative activity or cytotoxicity to human oral cancer cells (Seeram *et al.,* 2004), melanoma cells (Rodriguez *et al.,* 2002) and lung metastasis induced by B16F10 melanoma cells (Menon *et al.,* 1995). Also, the ethanolic extract contains gallic acid also exerts a cytotoxic activity against tumoral cells from leukemia, lung and prostate cancer origins (You and Park, 2010). In addition, sterols were found to inhibit tumor promotion in two-stage carcinogenesis in mice (Okwu, 2001). Steroid compounds are well known for their anticancer activity by inhibition of cancer cell proliferation, angiogenesis and induction of apoptosis (Kazlowska *et al.,* 2013).

Table 4. HPLC analysis of phenolic and flavonoid compounds of *Ficus sycamorus* fruits and leaves extracts

Compounds	Conc. µg/mg DW	
	Ethanolic extract of fruits	Ethanolic extract of leaves
Pyrogallol	-	1.97
Quinol	2.82	-
Gallic acid	1.72	4.72
Catechol	2.66	73.59
p-Hydroxy benzoic acid	-	45.38
Caffeine	11.87	4.61
Chlorgenic acid	-	272.94
Vanillic acid	23.02	41.44
Syringic acid	6.77	3.82
Caffeic acid	3.36	10.03
Vanillin	4.42	13.17
p-Coumaric acid	5.23	82.44
Ferulic acid	1.86	1.04
Benzoic acid	73.21	45.87
Rutin	1.33	366.89
Ellagic acid	-	3303.04
o- Coumaric acid	2.90	-
Salicylic acid	5.57	106.37
Flavonoids compounds		
Myricetin	20.31	141.24
Cinnamic acid	1.17	49.96
Quercitin	11.25	-
Neringenin	22.13	70.83
Rosmarinic acid	15.31	24.02
Kaempferol	108.72	20.40

Table 5. Antioxidant activity of *Ficus sycamorus* fruit and leaves extracts against DPPH method and reducing power methods

Conc. (µg/ml)	Fruits		Leaves	
	DPPH % in ethanolic extract	DPPH % in ethyl acetate extract	DPPH % in ethanolic extract	DPPH % in ethyl acetate extract
40	55.003	53.117	58.426	55.235
80	59.232	53.543	63.541	56.426
120	65.763	55.994	67.426	59.475
150	72.471	56.526	75.249	63.174
IC_{50} (µg/ml)	20.312	37.652	18.443	33.348
Reducing power activity (µg Gallic acid /100g DW	15.58±0.44	10.42±0.20	22.53±0.37	16.19±0.18

Table 6. Antibacterial activities of *Ficus sycamorus* fruit and leaves extracts against selected bacterial strains and fungus

Samples	Inhibition zone (mm)*					
	Gram positive bacteria		Gram negative bacteria		Fungus	
	S. aureus	*B. cereus*	*E. coli*	*S. typhimureum*	*A. niger*	*C. albicans*
Fruits ethanolic extract	8.18±0.22	9.25±0.16	15.37±0.36	17.11±0.25	9.71±0.19	10.32±0.42
Fruits ethyl acetate extract	6.14±0.44	8.14±0.35	12.72±0.24	14.31±0.17	6.43±0.18	8.75±0.33
Leaves ethanolic extract	10.46±0.42	12.61±0.29	17.82±0.51	19.31±0.11	12.60±0.33	13.21±0.16
Leaves ethyl acetate extract	8.11±0.13	10.41±0.15	14.09±0.16	15.21±0.52	7.32±0.26	9.34±0.41

Values are mean ± SD of three replicate analyses

Table 7. Anticancer activities of the ethanolic extract of fruits and leaves of *Ficus sycamores*

Concentrations (µg/ml)	Liver cell line (HepG2) Viability %		Colorectal adenocarcinoma (Caco-2) cell line Viability %		Breast cell line (MCF-7) Viability %	
	Fruits	Leaves	Fruits	Leaves	Fruits	Leaves
500	100	100	100	100	100	100
1000	94	97	99	99	93	91
1500	86	89	71	93	91	88
2000	79	82	68	88	85	78
3000	74	78	62	81	76	71
IC_{50} (µg/ml)	410	750	416	860	290	219

GC- mass analysis of fruits and leaves of Ficus sycomorus

Twilve compounds were identified in the ethanolic extract of fruits of *Ficus sycomorus* by GC-MS analysis. The active principles with their retention time (RT) molecular formula, molecular weight and % of peak area are present in Table (8) and Fig. (1). The prevailing compounds were Ambrosiol (8,9-dihydroxy-6,9a-dimethyl-3-methylidene-decahydro-azuleno[4,5-b]furan-2(3h)-one), N-Methyl-2-chloropyrrole, 4-Amino-3,5-di-2-pyridyl-4H-1,2,4-triazole, 2-Methylpyrrole-3-carbonitrile, 1,4-Cyclohexanedione, 2-Isopropyl-1,3-dioxolane, Phenyltrihydrosilane, Ethanone, 1-(2-methyl-1-cyclopenten-1-yl), Methyl ester of gibberellin A5, Benzene, 1,4-dimethyl-2,5-bis(1-methylethyl), 2,4-Di-tert-butylphenol and Phenyl 4-[bis(ethoxycarbonyl)but-3-ynyl]-2,3,4-trideoxy-.alpha.,L-glcero-pent-2-.

In addition, 29 compounds were identified in the ethanolic extract of *Ficus sycomorus* leaves by GC-MS analysis Table (9) and Fig. (2). The prevailing compounds were Gamma-Undecalactone, 2-Ethylcyclohexyl 3-chloropropanoate, 2-Ethylcyclohexyl bromoacetate, Aspidocarpine, 5-(1-Hydroxy-2-propanyl)-2-methylcyclohexanol, 2,7-Anhydro-l-galacto-heptulofuranose, 1,5-Naphthalenediol, Thiosulfuric Acid, Quinic acid, α-D-Glucopyranoside de α-D-glucopyranosyl-(1->3)-β-D-fructofuranosyle, hydrate, O-Benzyl-L-serine, 4-Allyl-2,6-dimethoxyphenol, [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester, D-chiro-Inositol, 3-O-(2-amino-4-(carboxyiminomethyl)amino)-2,3,4,6-tetradeoxy-, Neo Inositols, Psoralene, Phytol, acetate, 6-(3-Hydroxy-but-1-enyl)-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan-2-ol, Methyl Palmitate, Palmitic Acid, Methyl (11E,14E)-octadeca-11,14-dienoate, Methyl Linolenate, Phytol, Methyl isostearate, Linoleoyl chloride, cis-5,8,11,14,17-Eicosapentaenoic acid, Quercetin 7,3',4'-Trimethoxy and Lupeol. Similar results are reported by Romeh (2013) who found that the chemical analysis of vaporous from *F. sycomorus* leaves allowed identification of 1, 2 benzenedicarboxilic acid, diisooctyl ester (45.06%), n-Hexadecanoic acid (7.67%), 1H-pyrazole,4-Nitro (5.13%), 3-Hexen-1- ol, benzoate, Z (4.57%), oleic acid (4.30%), hexanedioic acid, bis (2-ethyl hexyl) ester (4.15%), methyl oleate (2.41%), 3- buten- 2-one, 4-(2, 6, 6- trimethyl-1-cyclohexen-1-yl) (2.08%), 9- octadecenoic acid (Z)- 2-hydroxy-1-(hydroxymethyl) ethyl ester (1.79%), benzene methanol (1.59%), Cycloheptasiloxane,tetradecamethyl (1.38%), Z, Z- 3, 13- octadecadien-1- ol (1.31%), 2-pentadecanone (1.27%), 1-methylbicyclo [4.1.0] heptanes 1-methylnorcarane (1.06%), L- linalool (1.04%), cyclohexene (1.03%) and methyl jasmonate (0.94%) as main parts.

Table 8. Compounds present in the *Ficus sycamorus* fruits using GC-MS analysis

Peak name	Retention time	Formula	MW	% Peak area
Ambrosiol (8,9-dihydroxy-6,9a-dimethyl-3-methylidene-decahydro-azuleno[4,5-b]furan-2(3h)-one)	2.497	$C_{15}H_{22}O_4$	266.34	30.64
N-Methyl-2-chloropyrrole	5.01	C_5H_6ClN	115.56	5.04
4-Amino-3,5-di-2-pyridyl-4H-1,2,4-triazole	4.293	$C_{12}H_{10}N_6$	238.25	5.07
2-Methylpyrrole-3-carbonitrile	7.031	$C_6H_6N_2$	112.13	7.06
1,4-Cyclohexanedione	7.675	$C_6H_8O_2$	106.13	4.71
2-Isopropyl-1,3-dioxolane	8.166	$C_6H_{12}O_2$	116.16	5.86
Phenyltrihydrosilane	8.255	$C_6H_5SiH_3$	108.21	3.91
Ethanone, 1-(2-methyl-1-cyclopenten-1-yl)	9.165	$C_8H_{12}O$	124.18	4.07
Methyl ester of gibberellin A5	9.261	$C_{20}H_{24}O_5$	344.40	4.38
Benzene, 1,4-dimethyl-2,5-bis(1-methylethyl)	14.81	$C_{14}H_{22}$	190.33	1.29
2,4-Di-tert-butylphenol	15.099	$C_{14}H_{22}O$	206.33	22.66
Phenyl 4-[bis(ethoxycarbonyl)but-3-ynyl]-2,3,4-trideoxy-.alpha.,L-glcero-pent-2-	22.443	$C_{21}H_{26}O_5$	374.00	5.31

Table 9. Compounds present in the *Ficus sycamorus* leaves using GC-MS analysis

Peak name	Retention time	Formula	MW	% Peak area
Gamma-Undecalactone	2.754	$C_1H_{22}O_2$	184.8	9.87
2-Ethylcyclohexyl 3-chloropropanoate	4.232	$C_{11}H_{19}ClO_3$	218.72	6.29
2-Ethylcyclohexyl bromoacetate	5.067	$C_{10}H_{17}BrO_2$	249.15	1.62
Aspidocarpine	5.432	$C_{22}H_{30}N_2O_3$	370.49	2.69
5-(1-Hydroxy-2-propanyl)-2-methylcyclohexanol	6.145	$C_{10}H_{20}O_2$	172.27	16.67
2,7-Anhydro-l-galacto-heptulofuranose	7.511	$C_7H_{12}O_6$	192.17	3.98
1,5-Naphthalenediol	7.824	$C_{10}H_8O$	160.17	2.02
Thiosulfuric Acid	9.241	$H_2S_2O_3$	114.13	5.12
Quinic acid	9.406	$C_7H_{12}O_6$	192.17	1.13
α-D-Glucopyranoside de α-D-glucopyranosyl-(1->3)-β-D-fructofuranosyle, hydrate	9.554	$C_{18}H_{34}O_{17}$	522.45	0.26
O-Benzyl-L-serine	9.667	$C_{10}H_{13}NO_3$	195.22	1.63
4-Allyl-2,6-dimethoxyphenol	9.989	$C_{11}H_{14}O_3$	194.23	0.61
[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	10.372	$C_{21}H_{38}O_2$	322.53	0.32
Nalidixic Acid	10.998	$C_{12}H_{12}N_2O_3$	232.24	0.72
D-chiro-Inositol, 3-O-(2-amino-4-((carboxyiminomethyl)amino)-2,3,4,6-tetradeoxy-	11.241	$C_{14}H_{25}N_3O_9$	379.363	1
Neo Inositols	11.372	$C_6H_{12}O_6$	180.16	3.4
Psoralene	11.902	$C_{11}H_6O_3$	186.17	25.32
Phytol, acetate	12.694	$C_{22}H_{42}O_2$	338.58	0.52
6-(3-Hydroxy-but-1-enyl)-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan-2-ol	12.85	$C_{13}H_{22}O_3$	226.31	0.28
Methyl Palmitate	13.241	$C_{17}H_{34}O_2$	270.46	3.88
Palmitic Acid	13.711	$C_{16}H_{32}O_2$	256.43	1.03
Methyl (11E,14E)-octadeca-11,14-dienoate	15.033	$C_{19}H_{34}O_2$	294.48	0.54
Methyl Linolenate	15.12	$C_{19}H_{32}O_2$	292.46	2.3
Phytol	15.268	$C_{20}H_{40}O$	296.54	1.62
Methyl isostearate	15.441	$C_{19}H_{38}O_2$	298.51	0.76
Linoleoyl chloride	15.572	$C_{18}H_{31}OCl$	298.89	0.37
cis-5,8,11,14,17-Eicosapentaenoic acid	15.798	$C_{20}H_{30}O_2$	302.45	0.87
Quercetin 7,3',4'-Trimethoxy	18.25	$C_{18}H_{16}O$	344.32	1.48
Lupeol	35.251	$C_{30}H_{50}O$	426.73	3.7

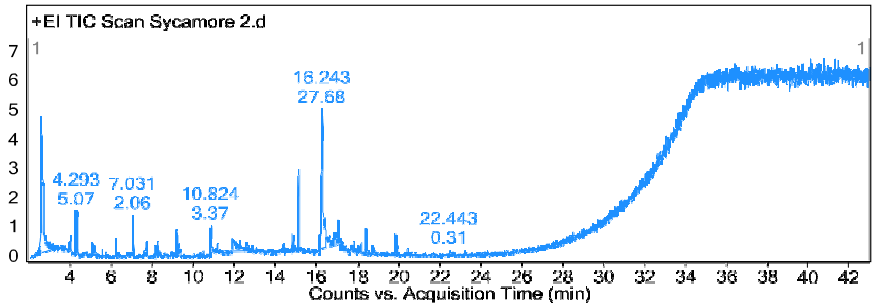

Fig. 1. GC-MS profiles of *Ficus sycomorus* fruits

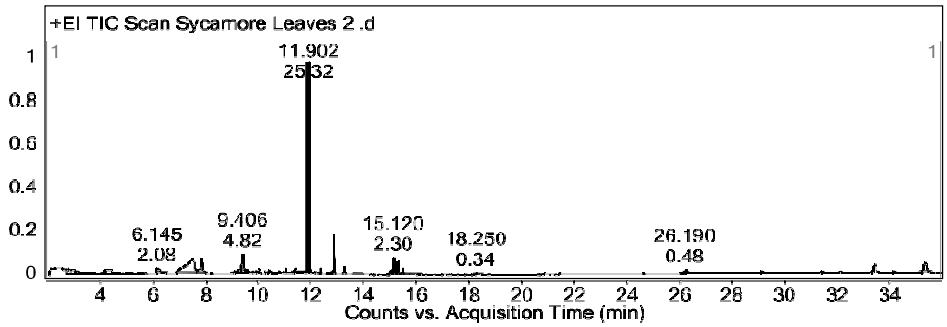

Fig. 2. GC-MS profiles of *Ficus sycomorus* leaves

GC- mass identified palmitic acid in leaves extract of ficus. Palmitic acid can be an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant (Praveen *et al.,* 2010). Also, Phytol, a bioactive principle, is also found to give effective preventive and therapeutic results against arthritis. The reactive oxygen species-promoting substances like phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases (Ogunlesi *et al.,* 2009). Phytol was noted to possess antibacterial activities against *Staphylococcus aureus* by inflicting harm to cell membrane as a result, there is a leakage of potassium ions from bacterial cells. In addition, some constituents are active for some diseases. Psoralen was reported to be inhibitory on tumor cells (Okuyama *et al.,* 1991).

Conclusions

This research is following a trend to effectively identify various compounds found in the ethanolic extract of fruits and leaves of *Ficus sycomorus* and finds its prophylactic role in designing and developing pharmacological drugs with fewer side effects. *In vitro* investigations in the present study provide substantial evidence that *Ficus sycomorus* leaves; an inedible waste product is a potent source of antioxidant, antimicrobial agent and anticancer activity thereby indicating its use as a value-added component for functional. In addition, *F. sycomorus* can be used as a source of natural antioxidants, which can be used as nutraceuticals to promote health, as preservatives to delay peroxidation of foods, and as flavoring for packed foods.

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