World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Investigation of phytochemicals and determination of compounds of *Phyllanthus emblica* Linn. and *Citrus limon* (L.) Burm. through proximate analysis and *In-vitro* antimicrobial activity against pathogenic fungi *Aspergillus flavus*

Irshad Ali Saudagar *, Farhat Jahan, Fatima Shirin, Ashish Tiwari, Arvindra Pal and Sushma Maravi

Genetics and Tree Improvement Division, Tropical Forest Research Institute, Jabalpur, 482021 Madhya Pradesh, India.

World Journal of Advanced Research and Reviews, 2022, 14(03), 203–213

Publication history: Received on 02 May 2022; revised on 05 June 2022; accepted on 07 June 2022

Article DOI: https://doi.org/10.30574/wjarr.2022.14.3.0526

Abstract

Phyllanthus emblica and *Citrus limon* are medicinal plants usually found throughout India and its neighboring countries used in traditional medicine. The present study amid to evaluate compounds present in fruits of these two species through proximate analysis method, qualitative phytochemical analysis of methanol and hexane extracts of *Phyllanthus emblica* and *Citrus limon*. Tannin, flavanoid, alkaloid, terpenoid, glycoside, saponin, carbohydrate, protein and resin, were screened in the present study. The plant extracts of these two species were evaluated for their antifungal activity against, *Aspergillus flavus*, by using agar well diffusion method. Results of the study showed that hexane extract of *Phyllanthus emblica* was able to provide a maximum zone of inhibition (8.60±0.95) against *Aspergillus flavus* on the third day of inoculation, whereas hexane extract of *Citrus limon* was given least zone of inhibition (3.07±0.27) on the third day at 50 mg/ml.The plant extracts utilized in the present study exhibit the presence of assorted active components, at the side of their antioxidant and antimicrobial activities which can be helpful against totally different infections or diseases. The methanol and binary compound fruit extracts possess effective repressive activity against the tested pathogens. Additional identification and purification of active chemical constituents from the crude extracts of such medicative plants are going to be useful to develop a drug against pathogenic fungi.

Keywords: Antimicrobial Activity; *Citrus Limon*; Proximate analysis; Phytochemicals; Phyllanthus *emblica*

1. Introduction

Phyllanthus emblica Linn. And *Citrus limon* (L.) Burm. are terribly helpful in our daily life. These domestically and medicinally important species are found in Central and southern India, Pakistan, the Mascarene Islands, South East Asia and Uzbekistan, and Fruits of these species can be employed in ayurvedic and herbal healing for treating several diseases (Rai *et al*., 2012; Thilaga *et al*., 2013; Khan, 2009) . Its content of high made amount of vitamin C, Flavanoid, Tannin, Poly-phenolic compounds and lycopene, which possess antioxidant activity.

Phyllanthus emblica commonly known as amla or Indian gooseberry belongs to the family Euphorbiaceae, is a deciduous tree that is employed in Chinese herbal medicines, Tibetan medicines and Ayurvedic medicines (IMP, 1997 and IHP, 1999). *Phyllanthus emblica* contains varied constituents like; alkaloids, benzenoid derivatives, diterpenes, furano-lactones, flavonoids and sterols, in several concentrations. It possesses medicative properties i.e. analgesic, antipyretic (James *et. al.*, 2004), metastatic tumor (Krishnaveni and Mirunalina, 2011), antioxidant (Scartezzini *et. al.*, 2006), antivenom (Makhija and Khamar,2010), antitussive (Nosalova*et. al.*, 2003), antimicrobial (Saeed and Tariq, 2007).), antitumour (Jeena *et. al.*, 2001), antiulcerogenic (Sairam, 2002), hypoprotective (Jeena*et. al.*,, 2000).), cytoprotective (Khanna and Nag, 1973) and antidiarrheal (Salud *et. al.*, 2007).

*Corresponding author: Irshad Ali Saudagar
Genetics and Tree Improvement Division, Tropical Forest Research Institute, Jabalpur, 482021 Madhya Pradesh, India.

Lemon (*Citrus limon*) is an edible fruit from a small tree or spreading bush in the rue family (*Rutaceae*). Juice is a common ingredient in a variety of desserts and pastries, including tarts and the classic Yankee lemon topping pie. The fruit's characteristic astringent flavour, whether fresh or preserved, is often utilized to enhance a variety of meals around the world. Lemonade, which is made with lemon, sugar, and water, is a popular summer beverage, and lemon juice is commonly added to tea in many cultures. Citric acid may make up 5% or more of the weight of lemon juice, which is also high in vitamin C and low in vitamin B, particularly thiamin, riboflavin, and niacin. Lemon juice is a crucial by-product .Among the necessary by-product of lemon is citric acid, citrate of lime, lemon oil, and pectin. Preparation of the oil, employed in perfumes, soap, and flavored extract, is a vital trade in Sicily.

Citrus essential oils (CEOs) are a mixture of volatile compounds consisting primarily of monoterpene hydrocarbons and are wide used in the food and pharmaceutical industries attributable to their antifungal activities. To face the challenge of growing awareness publicly and concern about food and health safety, studies concerning natural bio preservatives became the main focus of multidisciplinary research efforts. Within the previous decades, an outsized amount of literature has been published on the antifungal activity of CEOs. According to World Health Organization over 78% of the population depends on herbs used as ancient remedies for primary health-care (Diallo *et. al.*, 1999) medicative herbs are the potential source of therapeutic aids and have gained vital importance in the health-care system worldwide for each human and animals in pathological conditions and to take care of proper health. Indian herbal industries with significant research within the field of phytochemistry, pharmacognosy, pharmacology and clinical medical specialty have explored these herbs that are currently designed into various herbal formulations, which have entered the international pharmacopeia through the study and ancient medicine (Bhushan *et. al.*, 2004). Therefore, in present work an attempt was made to notice the presence of antifungal activities of fruits of *Phyllanthus emblica* and *Citrus limon* by a simple and unremarkably used agar well diffusion method.

Owing to the increase failure of chemotherapeutic and antibiotic resistance evidenced by morbific microorganism infectious pathogens, numerous medicative botanicals have been screened for their potential antimicrobial action (Kothari et. al., 2008 and Mujoriya, 2011). Plants have thousands of elements that are valuable sources of antibacterial compounds that are both new and biologically active. *Phyllanthus emblica* and *Citrus limon* have been shown to have antifungal properties against *Aspergillus niger* (Satish et. al., 2007). Where grisofulvin was applied as a standard antibiotic, ethanol and acetone extracts of fruits exhibited modest efficacy against *Fusarium equiseti* and *Candida spp* (Hossain et. al., 2012). *Phyllanthus emblica* plant methanolic extract had no antifungal effect against the phytopathogenic fungus *Aspergillus niger* F2723 (Bobbarala et. al., 2009).

2. Material and methods

2.1. Identification and Assortment of Plant Samples

Around one kilogram of fresh *Phyllanthus Emblica* and *Citrus limon* fruits were obtained from Jayantikunj, Rewa, M.P.'s district forest department nursery.

Fresh *Phyllanthus emblica* and *Citrus limon* fruits were washed under running water for 2-3 minutes, chopped into small pieces, air dried, then homogenized to a fine powder and stored in sealed glass bottles at 4°C in the refrigerator. They were then sorted and contaminants were removed. The plant's fruits were carefully air-dried for seven days. After the fruit samples had dried, they were ground into a coarse powder using a mechanical grinder, and the powder was stored in an extremely suited container for the extraction process.

2.2. Preparation of Plant Extracts

These plant extracts were ready by proceeding with consecutive cold maceration methodology using hexane and 1.50 gram of dried powder of plant material was soaked in 250 milliliter hexane for twenty-four hours at room temperature under shaking condition at 100 rpm. This solution was filtered with the help of Whatman No. 1 filter paper.

Then the filtrate was collected in petri-dishes of 15 centimeters and the solvent has been evaporated at room temperature. The dried extract was stored in 2 milliliter Eppendorf tube and after dilution, powder was used for antimicrobial assays. The filter cake was dried at room temperature and stored separately. The filter cake powder was consecutive resuspended in 250- milliliter methanol to prepare dried extract in every solvent. After extraction on every solvent, remained filter cake was dried and additionally used with the next solvent for extraction. All the dried extracts were stored in a refrigerator at 4°C. This residue is employed further for the analysis and antimicrobial study.

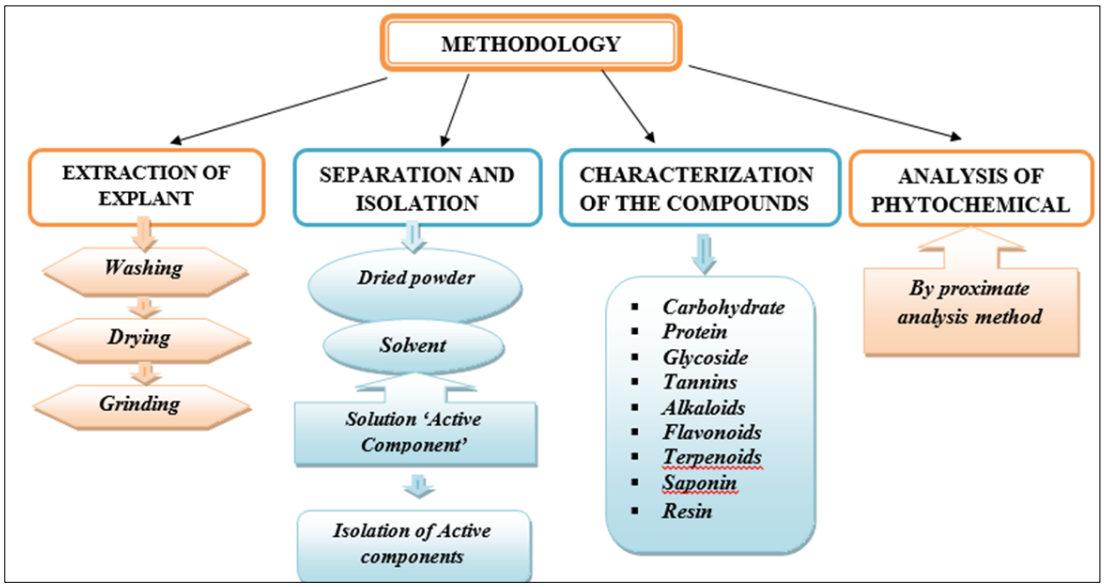


Figure 1 Overview of the approach

2.3. Collection of Pathogenic Fungi

Aspergillus flavus is a fungal pathogen that causes aspergillus ear and Kernel rot in plants. The fungus is usually found in soil as saprophyte, however it's a broad host range as an opportunistic pathogen. It causes vital losses in corn, peanuts, cottonseed, and tree nuts. The microorganism has collected from decayed peanuts by using ear-plug. *Aspergillus flavus* was collected in test tube and store at 4°C temperature.

2.4. Regeneration of Aspergillus flavus

For the determination of antifungal activity of methanol and hexane extracts of *Phyllanthus Emblica* and *Citrus limon*, pathogenic fungi *Aspergillus flavus* were grown to arrange the stock culture of fungi. Culture of *Aspergillus flavus* was cultivated within the slope agar medium of Sabouraud dextroglucose Agar (SDA). The whole rim of the tube mouth is passed on the flame. *Aspergillus flavus* culture is taken and separated on a sloping surface of agar medium. Incubated it at 25°C for two weeks. Megascopic study of fungal growth can be seen by their colonies. Colonies develops and exhibits a spectrum of overlapping characters.

2.5. Media Preparation

The fungal culture of *Aspergillus flavus* was grown in PDA culture medium. To create Potato Dextrose Agar (PDA), Titan Biotech Limited's potato dextrose agar was dissolved in 1.0 liter distilled water, yielding a medium known as potato dextrose agar. Before putting the medium into the Petri-dish, 30 milligrams of streptomycin were added to prevent bacterial infection.

2.6. Antifungal Activity through Agar Well Diffusion

The agar plates of the Potato dextroglucose Agar medium were prepared. Every plate was inoculated with an aliquot (0.1 milliliter) of the fungal suspension (103 spores/milliliter) that was spread equally on the plate, after 20 min, the wells with 6 millimeter diameter were made by using sterile cork borer and stuffed with test samples methanol and hexane extracts of *Phyllanthus Emblica* and *Citrus limon* of 50 milligram/milliliter. The positive and negative control plates were prepared using standard drug, fluconazole (10 microgram). All the plates were incubated at aerobically 26°C for 5-7 days so the diameter of the zone of inhibition was noted from time to time. Triplicates were applied for every extract against each of the test (Kanan and Rasha 2008).

The measurement of diameter of inhibition zone around each well was done and recorded at the end of the incubation period followed by third day and fifth day of the incubation. The extract concentration ready to inhibit microbic growth that was observed through the formation of an inhibition growth zone round the well.

2.7. Proximate Analysis

Titratable acidity was calculable by the tactic of the Association of Official Analytical Chemists (AOAC) (2005). A phenolphthalein indicator was used to titrate diluted extracts of *Phyllanthus emblica* and *Citrus limon* against 0.1 N sodium hydroxide (NaOH). The experiment was repeated three times. Citric acid in percent was used to report titratable acidity.

The AOAC technique was used to determine moisture and ash content. The fat content of the fruits was evaluated using a 5 gram extract in pre weighed thimbles using petroleum ether for extraction. The extraction took two hours to complete. Every measurement was repeated three times, with the mean shown in table 1.

Fat content was expressed in terms of proportion on a dry weight basis.The residue of theextract was utilized for the determination of crude fiber. Acidic and basic digestion was done using solvents, 0.255 N sulphuric acids (H2SO4) and 0.313 N sodium hydroxide (NaOH), respectively. After complete washing with boiling water, extract was kept in a muffle furnace for 30 minutes to destroy carbonous matter and loss in weight was calculated as crude fiber.

Protein content was calculable by the micro Kjeldahl method. Fresh fruits were digestible with a digestion mix and concentrated sulphuric acid. After its dilution, 10 milliliter of sodium hydroxide was added and distillation was done, the liquid was collected in 50 milliliter round shape flask containing 5- milliliter boric acid with a pair of drops of the mixed indicator until color of solution was changed. Then a volumetric analysis of distillate was allotted against normal acid (HCL) and concentration value was noted. Protein content was calculated.

Carbohydrate content was calculated in % by distinction technique [100 - ((%) moisture – (%) fat – (%) protein – (%) fiber)].

2.8. Qualitative Phytochemical Analysis of Plant Extracts

The methanol and hexane extracts of *Phyllanthus emblica* and *Citrus limon* were checked for the presence of tannin, flavanoid, alkaloid, terpenoid, glycoside, saponin, carbohydrate, protein and resin, consistent with normal protocols for detecting the presence of various chemical constitutes in each the extract of both plant.

2.9. Statistical Analysis

Mean and variance were calculated for every test. Data were subjected to statistical analysis of variance (ANOVA) technique exploitation Duncan's Multiple Range test (DMRT) and p values were considered significant at $p > 0.05$.

The % of inhibition was calculated by using the formula of Vincent.

$$\% \text{ of Inhibition} = (C-T) / C \times 100$$

Wherever C is that the growth in control in millimeter and T is growth in treatment in millimeters. All the experiments were applied in triplicate and average value was used for the interpretation of the results.

3. Results and discussion

3.1. Chemical Analysis

The nutritional value of *Phyllanthus emblica* and *Citrus limon* was determined by analyzing their proximate makeup. Fresh fruit moisture content is an incredibly important characteristic. Table 1 shows the percentage value of moisture content of *Phyllanthus Emblica* and *Citrus limon* fruits. It was discovered that the fruit of *Phyllanthus Emblica* had the highest moisture content (79.023%), while the fruit of *Citrus limon* had the lowest moisture content (78.867 percent). There was a considerable change in moisture content. Various researchers have previously reported moisture content of E. officinal is in the range of 79 to 86 percent (Singh et al., 2006; Ghori and Sethi, 1996; Garg, 2010).

Significant variation in ash content was conjointly noted that ranged from 2.24 to 3.08 %. Fruits of *Citrus limon* had the highest ash content (3.053 %) and the fruits of *Phyllanthus emblica* had the lowest (2.063 %). Protein contents among both species varied from 3.983 % in *Citrus limon* to 2.920% in *Phyllanthus emblica*. The data obtained was shows quite a similarity with numerous studies (Barthakur and Arnold, 1991; Singh *et. al.,* 1987; Pragati and Dhawan, 2001; Singh et al., 2006; Garg, 2010; Khan, 2009). Variation in fat contents of *Citrus limon* (1.207 %) was determined higher then *Phyllanthus emblica* (0.807 %) reported values of fat contents by Singh, (2012) and Singh, (2009), were within the range

of 0.10 % to 0.12 % on a fresh weight basis. Fruits are consulted for good supply of fibers. Crude fiber term usually includes polysaccharides cellulose, hemicelluloses, and lignin contents. Crude fiber was observed maximum in *Citrus limon* 17.013 % and minimum in *Phyllanthus emblica* 14.007 %. Results were comparable to the reported results by Pragati and Dhawan, (2001); Singh *et. al.,* (1987); and Khan, (2009). Total carbohydrate content was observed in *Phyllanthus emblica* at 70.320 % and maximum acidity as citric acid in *Citrus limon* at 26.769 %.

Table 1 Proximate analysis of the fruits of *Phyllanthus emblica* and *Citrus limon*

	Moisture (%)	**Ash (%)**	**Protein (%)**	**Fat (%)**	**Fiber (%)**	**Carbohydrate (%)**	**Acidity (%)**
PE	79.023	2.063	2.920	0.807	14.007	70.320	11.238
CL	78.867	3.053	3.983	1.207	17.013	54.807	26.769

Note: PE- Phyllanthus emblica, CL- Citrus limon

3.2. Estimation of Phytochemicals

The phytochemical analysis and estimation of the proportion of active components of the methanol and hexane extracts of *Phyllanthus emblica* and *Citrus limon* showed that the fruits of *Citrus limon* are made in alkaloids, flavonoids, tannins, phenols, and saponins (Singh *et. al.,* 2015). These secondary metabolites are responsible for their antibiotic activity. Flavonoids and tannins are phenolic compounds that act as primary antioxidants or scavengers of radicals (Khan and Sultana, 2006). Plant extracts show variability in their antimicrobial activities owing to their contents of active compounds. Numerous reports showed that alkaloids and flavonoids are the lead compounds that are liable for their antimicrobial activities (Cordell *et. al.,* 2001). whereas it's claimed that secondary metabolites such as tannins and a few phenolic compounds are classified as active antimicrobial compounds (Rojas *et. al.,* 1992), it prevents the micro-organisms through precipitating microbic protein, that act as an inhibitor for many fungi, yeasts, bacteria, and viruses (Prasad *et. al.,* 2008 and Chithrashree *et. al.,* 2014s).

An additional study was primarily geared toward determining whether the individual phytochemicals namely flavonoid, tannin, saponin, phenol present in fruits extract of *Phyllanthus emblica* and *Citrus limon* show any antimicrobial action toward the infectious agent used in the study. After the qualitative identification of the phytochemicals from the plant (Table 2), every phytochemical was tested in methanol and hexane extracts and so subjected to testing its antifungal activity against the pathogen.

Table 2 Qualitative analysis of phytochemicals present in different extracts

Phytochemicals ↓ → Samples	**PE Hexane**	**PE Methanol**	**CL Hexane**	**CL Methanol**
Tannin	-	+	+	-
Flavanoids	+	-	+	+
Alkaloid	-	+	-	+
Terpenoid	-	-	+	+
Glycoside	+	-	-	+
Saponin	-	+	-	-
Carbohydrates	+	+	-	+
Proteins	-	+	+	-
Resins	+	-	+	-

PE = Phyllanthus emblica, CL = Citrus limon, (+) = Presence, (−) = Absent

3.2.1. Test for carbohydrate (Telrandheet. al., 2006)

Molisch Test

A few drops of -naphthanol (20 percent in ethyl alcohol) were added to a 2 milliliter extract. The test tube was then filled with 1 millilitre of concentrated sulphuric acid (H2SO4). The presence of carbs is shown by the cherry-red violet ring at the confluence of the two layers. Except for the hexane extract of Citrus limon, carbohydrate has detected all of the extracts, which validates the work of Roghini R and Vijayalakshmi K. (2018).

3.2.2. Test for Protein (Telrandheet. al., 2010 and Culki, 1994)

A drop of copper sulphate (CuSO4) solution was gently added to 0.5 millilitre of liquid extract prepared with an equal volume of 1 percent sodium hydroxide (NaOH). The presence of protein was shown by the solution turning purple. The protein was found in *Phyllanthus emblica* methanol extract and *Citrus limon* hexane extract throughout the study. The findings were in contrast to those of Ashwin Rajkumar R et al., (2014), who found no protein content in citrus fruits in a study of six citrus family fruit extracts.

3.2.3. Test for Glycosides (Culki, 1994)

Keller-Killani test

1milliliter of glacial acetic acid (CH_3COOH) containing traces of ferric chloride ($FeCl_3$) and 1 ml of concentrated sulphuric acid were supplemented to the sample carefully. Achromatic color is formed at the junction of both the layers and then the upper layer turns bluish green, which indicates the presence of glycoside in the sample. Methanol extract of *Citrus limon* and hexane extract of *Phyllanthus emblica* were determined with the presence of glycoside content. DarshanDharajiya*et. al.,*2015 according to the absence of glycoside content in hexane extract of *E. officinalis*.

3.2.4. Test for Tannins (Evans, 2009 and Harborne, 1998)

To 1 milliliter of extract, 2 ml of ferric chloride ($FeCl_3$) was added. A navy or green black color indicates the presence of tannin. Tannin content was found present in methanol extract of *Phyllanthus emblica* and hexane extract of *Citrus limon*. Muhammad Majeed *et. al.,*(2009) determined tannins emblicanins from the fruits of *Phyllanthus emblica*. Roghini R and Vijayalakshmi K, (2018)reported that tannin content was absent in hexane extract of *Citrus limon* throughout their study.

3.2.5. Test for Alkaloids (Evans, 2009)

To 2 milliliter of each extract, 2 milliliters of concentrated hydrochloric acid (HCl) and a few drops of Mayer's reagent was added.A green or white precipitate indicates the presence of an alkaloid. Methanol extracts of *Phyllanthus emblica* and *Citrus limon* showed the presence of alkaloid contents, which supports several previous studies by Singh *et. al.,*(1987) ; Barthakur and Arnold, (1991); Pragati and Dhawan, (2001); . Dharajiya*et. al.,*(2015).

3.2.6. Test for Flavonoid (Culki, 1994 and Evans, 2009)

0.5 milliliter of sample solution was mixed with 2 ml of H2O and afterward with 0.15 milliliter of a 5 % sodium nitrite ($NaNO_2$) solution. after 6 minutes, 0.15 milliliter of a 10 % aluminum chloride ($AlCl_3$) solution was superimposed and allowed to face for 6 minutes, then 2 milliliter of 4% sodium hydroxide (NaOH) solution was added to the mixture. Water was added straightaway to bring the ultimatevolumeto5milliliter,then the mixturewas completely mixedandallowedtostandforanother 15minutes an appearance of light pink color indicates the presence of flavonoid. All the extracts were detected with presence of flavonoid content however it had been absent in methanol extract of *Phyllanthus emblica*.

3.2.7. Test for Terpenoid (Evans, 2009 and Harborne, 1998)

To 2milliliter of every extract 5 milliliter of chloroform and few drops of concentrated sulphuric acid (H_2SO_4) were rigorously added to make a layer. A sepia coloration formed within the interface indicates the presence of terpenoid. Terpenoid content was found absent in each extracts of *Phyllanthus emblica* and present in *Citrus limon*. Results was totally different to review of Khan, (2009); Kumar *et. al.,*(2012) who reported presence of terpenoid in *Phyllanthus emblica*.

3.2.8. Test for Saponin (Harborne, 1998)

Froth test

2 grams of the fine sample of both species is boiled with 10 milliliter of H_2O,filtered and mixed with 5 milliliter of distilled water and added some drops of olive oil and mixed it vigorously, then determined for the formation of emulsion. Methanol extract of *Phyllanthus emblica* was detected with presence of saponin content however an exceedingly different study by Okigbo RN, (2020) represents the presence of saponin content in *Citrus limon* fruits.

3.2.9. Test for Resin (Evans, 2009 and Harborne, 1998)

1 milliliter of the extracts was treated with a few drops of acetic anhydride followed by concentrated sulphuric acid (H_2SO_4). Color ranging from orange to yellow was noticed. Resins were presents in the hexane extract of fruits of each species.

3.3. *In vitro* Antifungal Test

Hexane and methanol extracts of *Phyllanthus emblica* and *Citrus limon* were found to possess antifungal potential in a different zone of inhibition against the check infective agent *Aspergillus flavus*. The results of the antifungal activity test of extract to *Aspergillus flavus* showed the presence of clear zones round the well.

Antifungal activity of hexane and methanol extracts of two medicative plants viz., *Phyllanthus emblica* and *Citrus limon* were evaluated through in *vitro* agar well diffusion method. As results of antifungal activity are shown in table 3, indicating that each the plant showed maximum antifungal activity against *Aspergillus flavus*. Results of the study showed that hexane extract of *Phyllanthus emblica* was able to provide a maximum zone of inhibition (8.60±0.95) against *Aspergillus flavus* on the third day of inoculation, whereas hexane extract of *Citrus limon* was given the least zone of inhibition (3.07±0.27) on the third day at 50 mg/ml. different extracts of *Phyllanthus emblica* and *Citrus limon* were show different repressive impact against fungi. As a per study, the extract of *Phyllanthus emblica* fruits was evidenced to be having significant antifungal activity against *C. albicans* and *A. niger* (Harborne P. R. *et. al.,* 2010).

Table 3 Antifungal activity (zone of inhibition in mm) of different extracts

Species	Extracts	Zone of Inhibition x Day	Zone of Inhibition (in mm)
Phyllanthus emblica	Methenolic	3rd day	4.17±0.55
	Hexanic	3rd day	8.60±0.95
Citrus limon	Methenolic	3rd day	7.07±0.09
	Hexanic	3rd day	3.07±0.27
Phyllanthus emblica	Methenolic	5th day	6.14±0.62
	Hexanic	5th day	6.23±0.52
Citrus limon	Methenolic	5th day	4.70±0.27
	Hexanic	5th day	7.80±0.60
C.D.			1.775
SE			0.58
SD			0.82
C.V.			16.812

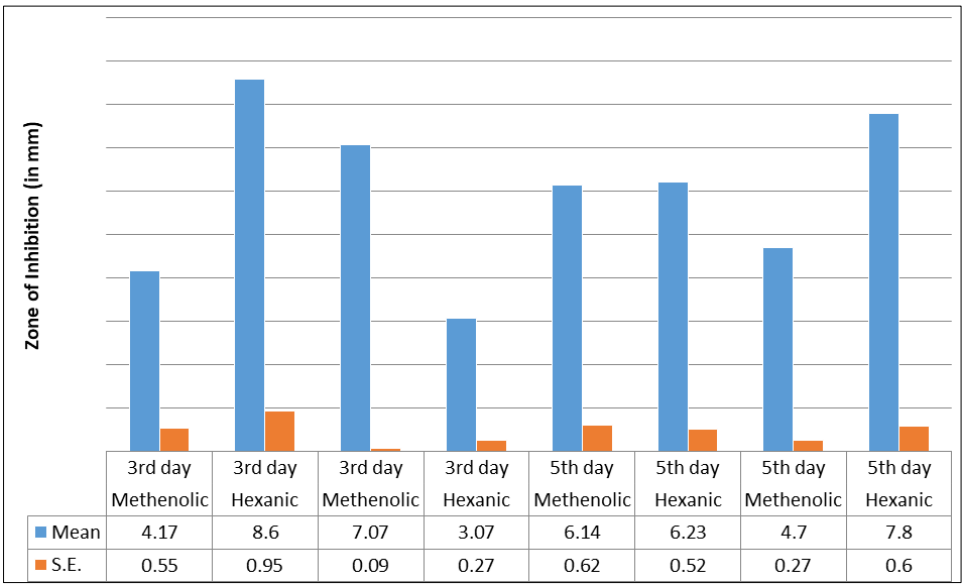
Note: Zone of Inhibition are given as means ± SD, CD= critical difference, SE= Standard error, SD= Standard deviation, CV= Coefficient of variance

Within the present study, hexane extract of *Phyllanthus emblica* showed inhibition against *Aspergillus flavus* (ZOI = 8.60±0.95) in only three days followed by methanol extract of *Phyllanthus emblica* (ZOI = 4.17±0.55). Methanol extract of *Citrus limon* was able to show antifungal activity against *Aspergillus flavus* (ZOI = 7.07±0.09) on the third day followed by hexane extract of *Citrus limon* (ZOI = 3.07±0.27). On fifth day hexane extract of *Citrus limon* was give maximal inhibition (ZOI = 7.80±0.60) followed by methanol extract of *Citrus limon* was able to show antifungal activity (ZOI

=4.70±0.27) and hexane extract of *Phyllanthus emblica* showed inhibition against *Aspergillus flavus* (ZOI=6.23±0.52) followed by methanol extract of *Phyllanthus emblica* (ZOI =6.14±0.62). In line with Mahmud *et. al.,* (2008), All the Aspergillus species employed in his study were slightly repressed by some extracts. In a previous study, crude extract of *V. negundo* fruits showed glorious antifungal activity against *Fusarium solani* and moderate restrictive activity against *Microsporum canis*. Whereas the same extract was proved ineffective against *A. flavus* and *C. albicans*.

Table 3 shows the findings of the chemical analysis of different phytochemicals found in the various extracts used in this investigation.Some work concerning chemical analysis of phytochemicals of *Phyllanthus emblica*, and *Citrus limon* was done in the recent past (Panda *et. al.,* 2009, Javale and Sabnis and Raphael 2012).The Presence of assorted photochemical in the extract can result in contribution to antifungal activity. Presence of phytochemicals in the higher amounts in extract, higher possibility of inhibitory action of the extract. There's need for additional quantitative chemical analysis of phytochemicals for the correct measurement of the phytochemicals. Results determined in the present study are found to be at par with the earlier reports (Fyhrquish, 2004). Nearly all the indentified compounds from fruits of both plants found active against microorganisms are aromatic or saturated organic compounds, they are most frequently obtained through initial ethanol or methanol extraction (Cowan,1999).

The data on the extent and mode of action for antifungal activity of specific compounds, present within the plant extracts, could lead to the eminent utilization of such natural compounds for the treatment of infections caused by infective fungi. For that there's need to determine such natural compounds from a wide range of medicative plants and to understand the mode of action of such chemical constituents (O.O.Agarry*et. al.,* 2005). This status of work regarding the antifungal activity of *Phyllanthus emblica* and *Citrus limon* is extremely restricted so this investigation could contribute to this field as a preliminary base. There is want for additional work on the antifungal activity of those plants using totally different pathogenic fungi for a suitable interpretation. Additional identification and purification of active chemical constituents from the crude extracts of such medicative plants are going to be useful to develop a drug against pathogenic fungi (S. Alemdar and S. Agaoglu, 2009).



	3rd day Methenolic	3rd day Hexanic	3rd day Methenolic	3rd day Hexanic	5th day Methenolic	5th day Hexanic	5th day Methenolic	5th day Hexanic
Mean	4.17	8.6	7.07	3.07	6.14	6.23	4.7	7.8
S.E.	0.55	0.95	0.09	0.27	0.62	0.52	0.27	0.6

Figure 2 Zone of Inhibition on action for antifungal activity of specific compounds

4. Conclusion

The well diffusion test is simple, easy to repeat, and inexpensive to browse and interpret, and it includes a connection to the reference microdilution test. It may be an alternative approach for antifungal drug susceptibleness testing of fungus in laboratories with limited resources. Antimicrobial activities were discovered in *Phyllanthus emblica* and Citrus limon, according to the study. The plant extracts used in this study have a variety of active components, as well as antioxidant and antibacterial properties that can aid in the treatment of a variety of infections and ailments. Methanol and binary compound fruit extracts had potent antimicrobial action against the pathogens examined. The findings of the investigation back up the plant's traditional knowledge claim.Plant extracts' antioxidant activity aids in the reduction of aerophilic stress. Additional pharmacologic assessments and the possibility of isolating the active

components from these plants are significant milestones toward future medicinal specialization for a variety of ailments.

Compliance with ethical standards

Acknowledgments

This research did not receive any form of grant from government or non-governmental organization.

Disclosure of conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

References

[1] Alemdar S, Agaoglu S. Investigation of in vitro antimicrobial activity of Aloe vera juice. Journal of animal and veterinary advances. 2009;8(1).

[2] Badoni HI, Sharma PR, Waheed SM, Singh SA. Phytochemical analyses and evaluation of antioxidant, antibacterial and toxic properties of Emblica officinalis and Terminalia bellirica fruit extracts. Asian J Pharm Clin Res. 2016;9(6):96-102.

[3] Barthakur NN, Arnold NP. Chemical analysis of the emblic (Phyllanthus emblica L.) and its potential as a food source. Scientia Horticulturae. 1991 Jun 1;47(1-2):99-105.

[4] Bobbarala V, Katikala PK, Naidu KC, Penumajji S. Antifungal activity of selected plant extracts against phytopathogenic fungi Aspergillus niger F2723. Indian Journal of Science and Technology. 2009 Apr 1;2(4):87-90.

[5] Chugh CA, Bharti D. Chemical characterization of antifungal constituents of Emblica officinalis. Allelopathy Journal. 2014 Oct 1;34(2).

[6] Cordell GA, Quinn-Beattie ML, Farnsworth NR. The potential of alkaloids in drug discovery. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2001 May;15(3):183-205.

[7] Cowan MM. Plant products as antimicrobial agents. Clinical microbiology reviews. 1999 Oct 1;12(4):564-82.

[8] Culki, I., 1994, Methodology of analysis of vegetable drug. Chemical industries branch, UNIDO, Romania. 1994; 24-27. 7. Evans W. C. Pharmacognosy. Saunders Publications. 15th Ed. 2007; 353- 355.

[9] Dahiya S, Dhawan SS. Effect of drying methods on nutritional composition of dehydrated aonla fruit (Emblica officinalis Garten) during storage. Plant Foods for Human Nutrition. 2003 Sep;58(3):1-9.

[10] De Silva T. medicinal plants in developing countries. Medicinal plants for forest conservation and health care. 1997;92:34.

[11] Diallo D, Hveem B, Mahmoud MA, Berge G, Paulsen BS, Maiga A. An ethnobotanical survey of herbal drugs of Gourma district, Mali. Pharmaceutical Biology. 1999 Jan 1;37(1):80-91.

[12] Fyhrquish, P., Mwasumbi, L., Haeggstrom, C., Vuorela, H., 2004, Antifungal activity of selected spp. of collected in Tanzania.. 42: 308-317.

[13] Garg A. Studies on processing and development of carbonated spiced RTS drink from Bitter Gourd and Amla fruit. Processed Food Industry. 2010;13(11):23-6.

[14] Ghorai K, Sethi V. Varietal suitability of Amla ('Desi'and'Banarasi') fruits for storage and preservation. Indian Food Packer. 1996;50:11-8.

[15] Gutiérrez SP, Sánchez MA, González CP, García LA. Antidiarrhoeal activity of different plants used in traditional medicine. African Journal of Biotechnology. 2007;6(25).

[16] Harborne P. R. Tharkar, A. U. Tatiya, S. J. Surana, N. S. Bhajipale and S. R. Harbrone, 2009, J. B., 1973, Phytochemicals methods. London. Chapman and Hill.

[17] Hossain MM, Mazumder K, Hossen SM, Tanmy TT, Rashid MJ. In vitro studies on antibacterial and antifungal activities of Emblica officinalis. International Journal of Pharmaceutical Sciences and Research. 2012 Apr 1;3(4):1124-7.

[18] Indian Herbal Pharmacopoeia, 1999, Part 2, pp. 50. Regional Research Lab (CSIR) and Indian Drug Manufacturers Association, Mumbai, India

[19] Indian Medicinal Plants-A compendium of 500 species, Part 3, 1997, pp. 256-263. Orient Longman Publications, Mumbai, India.

[20] Jose JK, Kuttan G, Kuttan R. Antitumour activity of Emblica officinalis. Journal of Ethnopharmacology. 2001 May 1;75(2-3):65-9.

[21] Jose JK, Kuttan R. Hepatoprotective activity of Emblica officinalis and Chyavanaprash. Journal of Ethnopharmacology. 2000 Sep 1;72(1-2):135-40.

[22] Kanan, G. J. and Rasha, A. A. N., 2008, In vitro Antifungal Activities of Various Plant Crude Extracts and Fractions Against Citrus post-harvest Disease Agent Penicilliumdigitatum Volume 1, Number 3, September. ISSN 1995-6673 Pages 89 - 99 Jordan Journal of Biological Sciences

[23] Kaushik, R. D., Sharma, G. and Arora, C., 2004, Biocidal potential of leaf extracts of Vitex negundo L. Allelopathy Journal 14: 227-234. .

[24] Khan TH, Sultana S. Apigenin induces apoptosis in Hep G2 cells: possible role of TNF-α and IFN-γ. Toxicology. 2006 Jan 16;217(2-3):206-12.

[25] Khan, K. H., 2009, Role of Emblica offincinalis in medicine – A Review. Botany Research International 2: 218- 228.

[26] Khanna, P. and Nag, T. N., 1973, Isolation, Identification and Screening of Phyllemblin from Emblica officinalis Gaertn. tissue culture. The Indian Journal of Pharmacy 35: 23-25.

[27] Kothari S, Jain AK, Mehta SC, Tonpay SD. Effect of fresh Triticum aestivum grass juice on lipid profile of normal rats. Indian journal of pharmacology. 2008 Oct;40(5):235.

[28] Krishnaveni M, Mirunalini S. Amla–The role of ayurvedic therapeutic herb in cancer. Asian journal of pharmaceutical and clinical research. 2011;4(3):13-7.

[29] Kumar KS, Bhowmik D, Dutta A, Yadav AP, Paswan S, Srivastava S, Deb L. Recent trends in potential traditional Indian herbs Emblica officinalis and its medicinal importance. Journal of Pharmacognosy and Phytochemistry. 2012 May 1;1(1):18-28.

[30] Majeed M, Bhat B, Jadhav AN, Srivastava JS, Nagabhushanam K. Ascorbic Acid and Tannins from Emblica officinalis Gaertn. Fruits A Revisit. Journal of Agricultural and Food Chemistry. 2009 Jan 14;57(1):220-5.

[31] Makhija IK, Khamar D. Anti-snake venom properties of medicinal plants. Der Pharmacia Lettre. 2010;2(5):399-411.

[32] Md Noh MF, Gunasegavan RD, Mustafa Khalid N, Balasubramaniam V, Mustar S, Abd Rashed A. Recent techniques in nutrient analysis for food composition database. Molecules. 2020 Jan;25(19):4567.

[33] Mujoriya R, Bodla RB. A study on wheat grass and its nutritional value. Food Science and Quality Management. 2011;2:1-8

[34] Nosal'ova G, Mokrý J, Hassan KT. Antitussive activity of the fruit extract of Emblica officinalis Gaertn.(Euphorbiaceae). Phytomedicine. 2003 Jan 1;10(6-7):583-9.

[35] Okigbo RN, Ezebo RO, Okoli SI. Phytochemical analysis of Xylopia aethiopica (Dun), Citrus limon (L.) and Allium sativum (L.) extracts and their effect on selected human pathogens. Adv Med Plant Res. 2020;8:35-42.

[36] Olaleye MT, Bello-Michael CO. Comparative antimicrobial activities of Aloe vera gel and leaf. African journal of biotechnology. 2005;4(12).

[37] Patwardhan B, Vaidya AD, Chorghade M. Ayurveda and natural products drug discovery. Current science. 2004 Mar 25:789-99.

[38] Prasad NR. Viswanathan. S., Renuka Devi, J., Vijayashree Nayak., Sweth, VC, Archana Parathasarathy, N and Johanna Rajkumar. 2008:268-70.

[39] Rai N, Tiwari L, Sharma RK, Verma AK. Pharmaco-botanical profile on Emblica officinalis Gaertn. A Pharmacopoeial Herbal Drug. STM Journals. 2012;1(1):29-41.

[40] Rojas A, Hernandez L, Pereda-Miranda R, Mata R. Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants. Journal of ethnopharmacology. 1992 Jan 1;35(3):275-83.

[41] Panda S. K., Thatoi H. N. and Dutta S. K., Antibacterial activity and phytochemicalscreening of leaf and bark extracts of Vitex negundo L. from similipal biosphereereserve, Orissa, J. Med. Plant. Res., 2009, 3(4), 294-300

[42] Saeed S, Tariq P. Antibacterial activities of Emblica officinalis and Coriandrum sativum against Gram negative urinary pathogens. Pakistan journal of pharmaceutical sciences. 2007 Jan 1;20(1):32-5.

[43] Sairam KC, Rao CV, Babu MD, Kumar KV, Agrawal VK, Goel RK. Antiulcerogenic effect of methanolic extract of Emblica officinalis: an experimental study. Journal of ethnopharmacology. 2002 Sep 1;82(1):1-9.

[44] Satish S, Mohana DC, Ranhavendra MP, Raveesha KA. Antifungal activity of some plant extracts against important seed borne pathogens of Aspergillus sp. Journal of Agricultural technology. 2007 Jan 1;3(1):109-19.

[45] Scartezzini P, Antognoni F, Raggi MA, Poli F, Sabbioni C. Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparation of Emblica officinalis Gaertn. Journal of ethnopharmacology. 2006 Mar 8;104(1-2):113-8.

[46] Singh DI. Aonla: Production, Handling and Processing. Westville Publishing House; 2012.

[47] Singh PL, Ganesan S, Singh S. Drying study of amla (Emblica officinalis Gaertn.) in a solar tunnel dryer. Indian Food Packer. 2006;60(1):47.

[48] Singh R, Dashora LK, Upadhyay B. Effect of pre-drying treatments and drying methods on physico-nutritional quality of dehydrated aonla shreds. Indian Food Packer. 2006;60(3):57.

[49] Singh, B. P., Singh, I. P., Singh, S. P. and Kumar, K. A., 1987, Physico-chemical composition of different cultivars of Aonla. Indian Food Packer 41: 7-10.

[50] Taherian AR, Fustier P, Ramaswamy HS. Effect of added oil and modified starch on rheological properties, droplet size distribution, opacity and stability of beverage cloud emulsions. Journal of Food Engineering. 2006 Dec 1;77(3):687-96.

[51] Thilaga S, Largia MJ, Parameswari A, Nair RR, Ganesh D. High frequency somatic embryogenesis from leaf tissue of'Emblica officinalis' Gaertn.-A high valued tree for non-timber forest products. Australian Journal of Crop Science. 2013 Sep 1;7(10):1480-7.