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Phytochemical Screening and Antioxidant Activity of Few Medicinal Plants

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Abstract: In the present study, methanolic extract of leaves of *Piper nigrum* L., *Citrus maxima* (Burm.f.) Merr. and *Phyllanthus emblica* L. was prepared and the extracts were tested for phytochemical analysis and antioxidant assay. Alkaloids, phenols, tannins, flavonoids and amino acids were present in selected three leaves extract. Maximum metabolites are present in *Phyllanthus emblica* L. The quantitative phytochemical tests exhibited maximum phenol and tannin content in *Phyllanthus emblica* L. than *Piper nigrum* L. and *Citrus maxima* (Burm.f.) Merr. The antioxidant activity was accessed using DPPH Assay for the selected leaves. The result showed that *Phyllanthus emblica* L. has strong antioxidant activity followed by *Piper nigrum* L. and *Citrus maxima* (Burm.f.) Merr.

Keywords: *Piper nigrum* L., *Citrus maxima* (Burm.f.) Merr., *Phyllanthus emblica* L., Antioxidant, Phytochemical, DPPH assay

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Introduction

Medicinal plants are valuable source of organic materials for maintaining human health. Due to the expensive cost and increased side effects of pharmaceuticals made chemically, there is currently a great deal of interest in the research sector for natural plant products used for pharmacological reasons (Gurupriya *et al.*, 2017). Medicinal plants are readily accessible worldwide and are receiving increased attention due to their potential to improve human society in numerous ways, including treatment of various diseases. They function as both therapeutic agents and vital raw materials in the production of both conventional and contemporary medicine. The use of medicinal plants, including their fresh or dried components, whole, chopped, powdered, or in more advanced forms usually produced through extraction with various solvents, plays a significant role and forms the backbone of most traditional medical systems (Showmiya and Ananthi, 2018).

Piper nigrum L., commonly known as "Black

pepper", is a flowering vine belonging to the Piperaceae family that is grown for its fruit. The fruit is typically dried and used as a seasoning and spice. The fruits and roots have a wide range of medical applications. They can be used as a counter-irritant and analgesic when applied locally for muscular pains and inflammation, as snuff in cases of coma and drowsiness, and internally as a carminative (Ganesh *et al.,* 2014). *Citrus maxima* (Burm.f.) Merr. commonly referred to as shaddock, chakkotra or pomelo, is a perennial shrub native to the Indian subcontinent. It belongs to the family Rutaceae. Among the citrus fruits, it is the largest. The nutritional value and pharmacological characteristics of *C. maxima* (Burm.f.) Merr. are revealed by its leaves, flowers, rind, fruits, roots, and bark, emphasizing its application in traditional medicine as a remedy for a range of ailments (Flama *et al.*, 2022). *Phyllanthus emblica* L. also called 'Indian gooseberry' is one of the richest sources of vitamin C and is widely distributed in deciduous forests. It is ubiquitous throughout India's tropical and subtropical regions. In traditional Chinese and Indian (Ayurvedic) medical systems, various parts of the *Phyllanthus emblica* L. plant are employed as conventional remedies for a variety of diseases (Kenneth *et al*., 2018).

Medicinal plants have organic compounds called bioactive substances, such as tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids that have specific physiological effects on humans. Secondary metabolites are incredibly diverse chemical and taxonomically diverse substances with unknown functions. They find extensive applications in scientific research, veterinary medicine, agriculture, human therapy, and numerous other fields. *In vitro* studies have demonstrated the inhibitory effects of several phytochemicals from various chemical classes on a wide range of microorganisms (Yadav and Agarwala, 2011).

Antioxidant action of naturally occurring compounds in higher plants has long been recognized. Oxygen-containing free radicals in biological systems and their potential involvement as causative agents in the aetiology of various chronic diseases have garnered increased attention in recent times. As a result, interest is being drawn to the molecular defense mechanisms that naturally occurring antioxidants perform in the cells of the organisms that contain them (Larson, 1988). The main objective of the present study was to find out the bioactive constituents present in the methanol leaf extract of *Piper nigrum* L.*, Citrus maxima* (Burm.f.) Merr. and *Phyllanthus emblica* L. and to evaluate the antioxidant activity of these plant extracts.

Materials and Methods

Plant collection:

Leaves of *Piper nigrum* L., *Citrus maxima* (Burm.f.) Merr. and *Phyllanthus emblica* L. were collected from in and around Palakkad and Malappuram district, Kerala, India. The leaves were authenticated by Dr. Sreekumar V. B., Principal Scientist, KSCSTE- Kerala Forest Research Institute, Thrissur, India. Healthy leaves were collected and washed with distilled water to remove dust and contaminants. Once dried, the leaves were ground into fine powder using an electronic blender.

Plant extraction:

The dried powdered plant material was extracted with methanol using the standard Soxhlet extraction method for 72 h. Later, the extracts were filtered using Whatman filter paper and dried in a rotary evaporator at low pressure to produce solid or semisolid residues. The remnants were lyophilized to get dry solid mass.

Preparation of leaf extract:

The powdered leaves were mixed with methanol at a concentration of 1:10 (w/v). Methanolic extraction process was carried out at room temperature and was left for shaking for 24 h in an orbital shaker. The suspension obtained was filtered using a 114 Whatman filter paper and the filtrate was collected.

Qualitative Phytochemical Analysis:

The phytochemical examinations were carried out for all the plant extracts using standard procedure (Evans, 2009).

Detection of Alkaloids:

Mayer's Test: 1 ml of the filtrates were treated with few drops of Mayer's reagent (Potassium Mercuric Iodide). Formation of fairly yellow coloured precipitate showed the presence of alkaloids.

Dragendroff's Test: 1 ml of the filtrates were treated with few drops of Dragendroff"s reagent (solution of Potassium Bismuth Iodide). Formation of reddish precipitate confirmed the presence of alkaloids.

Detection of Carbohydrates:

Fehling's Test: 1 ml of the filtrates were treated with dilute Hydrochloric acid, neutralized with alkali and then heated with Fehling's A and Fehling's B solutions. Presence of reducing sugars is confirmed with appearance of red precipitate.

Detection of Glycosides:

Legal's Test: 1 ml of the extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Pink colour changed to blood red colour, which confirmed the presence of cardiac glycosides.

Detection of Saponins:

Foam Test: 0.5 g of the extract were added to 2 ml of water and shaken well. The presence of saponins were confirmed if the foam produced persisted for 10 min.

Detection of Phytosterols:

Salkowski's Test: 1 ml of the extracts were treated with chloroform and filtered. 3-4 drops of concentrated Sulphuric acid was added, shaken well and kept undisturbed. Formation of golden yellow colour showed the presence of phytosterols (Triterpenes).

Detection of Phenols;

Folin-Ciocalteu Test: 1 ml of the plant extract was treated with sodium carbonate and 2-3 drops of phenol reagent. Presence of phenol was indicated by the appearance of blue colour.

Detection of Tannins:

Gelatin Test: 1% gelatin solution containing sodium chloride was added to 1 ml of extract. With the formation of white precipitate, presence of tannins was confirmed.

Detection of Flavonoids:

Alkaline Reagent Test: To 1 ml of extract few drops of sodium hydroxide solution was added. Intense yellow colour was formed and on adding dilute acid became colourless which confirmed the presence of flavanoids.

Detection of Proteins and Amino acids:

Xanthoproteic Test: A few drops of conc. Nitric acid were added to the extract. The development of yellow colour indicated the presence of proteins

Ninhydrin test: 0.25% w/v ninhydrin reagent was added to the extract and boiled for few minutes. The appearance of blue colour indicated the presence of amino acid.

Detection of Terpenes:

Chloroform was added to the extracts and filtered. A few drops of conc. sulfuric acid were added to the filtrate immediately. The presence of terpenes was indicated by the formation of a reddish brown precipitate.

Quantitative Phytochemical Analysis:

Estimation of Total Phenols: Folin-Ciocalteu Reagent (FCR) was used to determine total phenol content. In an alkaline medium, phenols combine with phosphomolybidic acid in the Folin-Ciocalteu Reagent to form molybdenum blue. To prepare a working standard solution, dissolved 100 mg of catechol in 100 ml of distilled water and diluted 10 ml of the stock in 100 ml of distilled water. Take 0.1 ml of sample and make up to 1 ml with distilled water. 0.5 ml Folin-Ciocalteu reagent was added. 2 ml of 20% sodium carbonate solution was added after 5 min and make up to 10 ml with distilled water. Incubated at room temperature for 10 min. Spectrophotometer was used to measure the absorbance of the sample at 650 nm with

respect to the blank. Catechol was used as a standard in the construction of a calibration curve, and the total phenolic content of the extract is reported in milligrams of catechol per gram of dry weight (Malik and Singh, 1980)

Estimation of Total Flavonoids: By using Cameron *et al.* (1943) method, flavanoids in plant extract was estimated. In an acidic medium, flavanoids react with the vanillin regent and produce a yellow colour. Prepared a working standard solution by dissolving 100 mg of catechin (100 µg/ml) in 100 ml of distilled water and diluted 1 ml of the stock in 10 ml of distilled water. Pipette 0.1 ml of the extract into the test tube and make up to 3 ml using distilled water. Subsequently added 4 ml of vanillin reagent and placed the test tubes in boiling water bath for 15 min. Varying concentration of the standards were treated similarly to obtain standard curve. Absorbance was measured spectrophotometrically at 415 nm. The amount of flavanoids in each sample is expressed as µg flavanoids/ g sample.

Estimation of Total Tannins: Tannin estimation was carried out using Vanillin hydrochloride method. To make 1 ml, 0.1 ml of the extract was diluted with distilled water. Then 5 ml of vanillin hydrochloride reagent (Equal volume of 8% HCL in methanol and 4% vanillin in methanol) was mixed with the extract. After 20 min at room temperature incubation, the sample was measured at 500 nm. Catechin was used as the standard to prepare the standard graph. Tannin content of the sample is represented as catechin equivalents (Hangerman, 2002).

Estimation of Saponins: 50 µl of plant extract was mixed with 250 µl of distilled water. Vanillin reagent (800 mg of vanillin mixed with 10 ml of 99.5% ethanol) was added to the above solution. Afterwards, 2.5 ml of 72% sulphuric acid was added and thoroughly mixed. For 10 min, this solution was retained in a water bath at 60°C. Following a 10 min cooling period in ice cold water, the absorbance was determined at 544 nm. Based on a standard curve the values are expressed as diosgenin equivalents (Hiai *et al*., 1976).

Antioxidant Activity:

DPPH Assay:

Prepared 0.1 mM DPPH solution in ethanol. To 3 ml of the extract solution, added 1 ml of the above solution at various concentrations (20-100 mg/ml). At room temperature, incubated for 30 min. Make a blank without extract and determined the absorbance at 517 nm. Ascorbic acid standard is prepared at different concentrations (20 to 100 mg/ml).

The following equation was used to determine the DPPH radical's scavenging capacity:

DPPH Scavenged (%) = [(A control- A test) / A control] *100

Results

The preliminary phytochemical screening of the methanolic extract of *Piper nigrum* L., *Citrus maxima* (Burm.f.) Merr. and *Phyllanthus emblica* L. are depicted in Table 1. In the present study, the qualitative and quantitative phytochemical screening of methanolic *Piper nigrum* L. extract were assessed for the presence of primary and secondary metabolites. The results showed the presence of alkaloids, phenols, tannins, flavanoids, proteins and amino acids while carbohydrates, glycosides, saponins, phytosterols, terpenes were absent. Total phenols, flavanoids and tannins were quantified. Alkaloids, carbohydrates, phenols, tannins, flavanoids and amino acids were the active phytochemical classes showed in the phytochemical screening tests for *Citrus maxima* methanol extract. The extract lacked glycosides, phytosterols, saponins, proteins and terpenes. On the basis of phytochemical analysis the methanolic extract of *Phyllanthus emblica* L. revealed the presence of alkaloids, glycosides, saponins, phytosterols, phenols, tannins, flavanoids aminoacids and terpenes whereas carbohydrates and proteins were absent.

Quantitative estimation of Total Phenols, Flavonoids, Tannins, and Saponins from methanol leaf extracts are depicted in Table 2. Total phenol content in *Piper nigrum* L., *Citrus*

Table 1: Qualitative phytochemical screening of methanolic leaf extracts of *Piper nigrum* L., *Citrus maxima* (Burm.f.) Merr. and *Phyllanthus emblica* L.

S. No.	Phytochemicals	Test Name	Observation	*Piper nigrum* L.	*Citrus maxima* (Burm.f.) Merr.	*Phyllanthus emblica* L.
1.	Alkaloids	Mayer's Test	Yellow precipitate	+	+	+
		Dragendroff's Test	Red precipitate	+	+	+
2.	Carbohydrates	Fehling's Test	Red precipitate	-	+	-
3.	Glycosides	Legal's Test	Blood red colour	-	-	+
4.	Saponins	Foam Test	Foam produced persists for 10 minutes	-	-	+
5.	Phytosterols	Salkowski's Test	Golden yellow colour	-	-	+
6.	Phenols	Folin-Ciocalteu Test	Blue colour	+	+	+
7.	Tannins	Gelatin Test	White precipitate	+	+	+
8.	Flavanoids	Alkaline Reagent Test	Intense yellow colour, becomes colourless on addition of dilute acid	+	+	+
9.	Proteins	Xanthoproteic Test	Yellow colour	+	-	-
10.	Amino acids	Ninhydrin Test	Blue colour	+	+	+
11.	Terpenes	Copper Acetate Test	Emerald green colour	-	-	+

Table 2: Comparative Quantitative estimation of Total Phenols, Flavanoids, Tannins, and Saponins of three selected leaves in methanol extract

Plants	Extracts	Total phenolics (mg/ml of extract)	Total flavonoids (mg/ml of extract)	Total tannins (mg/ml of extract)	Total saponins (µg/ml)
Piper nigrum L.	Methanol	0.75 ± 0.0163	Trace amounts	0.975±0.2003	-
Citrus maxima (Burm.f.) Merr	Methanol	0.5123±0.0021	Trace amounts	0.758 ± 0.011	-
Phyllanthus emblica	Methanol	1.6430 ± 0.000	Trace amounts	2.091 ±0.058	3.936±0.0

Results were expressed as mean ± standard deviation

maxima (Burm.f.) Merr., *Phyllanthus emblica* L. extracts are found to be 0.75±0.0163 mg/ml**,** 0.5123±0.021 mg/ml, and 1.6430±0.00 mg/ml, respectively. It was discovered that the *Piper nigrum* L. extract showed highest phenol content. The extracts of *Piper nigrum* L., *Citrus maxima* (Burm.f.) Merr., and *Phyllanthus emblica* L. only contain trace amounts of flavonoids overall. The total tannin content in the extracts were evaluated as 0.975±0.2003 mg/ml (*Piper nigrum* L.), 0.758±0.011 mg/ml (*Citrus maxima* (Burm.f.) Merr.), and 2.091±0.058 mg/ml (*Phyllanthus*

emblica L.). Quantitative phytochemical analysis revealed that tannin concentration was higher in *Phyllanthus emblica* L. The total saponin content in *Phyllanthus emblica* L. extract is determined and found to be 33.936±0.0 mg/ml.

The antioxidant activity of the plant extract are evaluated using DPPH (2,2-diphenyl-1-picryl-hydrazyl) assay by calculating the scavenging capacity of DPPH present in the plant extracts. The scavenging activity of free radicals can assesed quickly and effectively with the DPPH test. To ascertain the antioxidant activity, the percentage of inhibition was calculated. IC_{50} value also determined to valuate the concentration of sample that can scavenge 50% of free radical. The greater antioxidant activity is indicated by a lower IC_{50} value. In the present study, the lowest IC_{50} was found in *Phyllanthus emblica* L. with a value of 8.8 µg and highest in *Citrus maxima* (Burm.f.) Merr. with 366.95. The *Piper nigrum* L. has a IC_{50} value of 40.07. This showed that *Phyllanthus emblica* L. has a higher antioxidant property than the other samples under study.

Discussion

Alkaloids, flavanoids, phenols, tannins, glycosides, saponins, terpenes etc. are significant compounds with a variety of biological functions. By identifying the phytochemicals in a plant, one can anticipate its pharmacological activity. Even though phytochemicals are now determined using a variety of contemporary methods, traditional qualitative assays are still widely used for plants' initial phytochemical screening (Junaid and Patil, 2020). The findings of the current study indicated that *Piper nigrum* L. leaf extract included a number of phytochemicals. The phytochemicals such as alkaloids, phenols, tannins, flavonoids and proteins are present in *Piper nigrum* L. extract.

The given results are in accordance with the study of Muhammad *et al.* (2018) who reported that the extract contain alkaloids. Alkaloids are a broad class of nitrogenous compounds that are widely used as anesthetics, central nervous stimulants, and cancer chemotherapeutic agents. It is well known that alkaloids regulate development and have certain metabolic functions in living systems. The extract also contains flavanoids, which have high anticancer properties and prevent oxidative cell damage. Flavanoids are a powerful water-soluble antioxidant and free radical scavenger.

According to the qualitative analysis of the plant extract and phytochemical screening alkaloids, phenols, tannins, and flavanoids were abundant in the leaves of the plant. In addition to their physiological activities, they have therapeutic properties (Ajmad *et al.*, 2023). Preliminary phytochemical test done in this study revealed that the methanolic extract of *Piper nigrum* L. contained phenols, tannins, alkaloids, and other secondary metabolites. According to the phytochemical screening and quantitative estimation of extract, alkaloids, flavanoids, tannins, phenols and saponins were abundant in the leaves. Both medicinal and physiological activity were displayed by plants (Gayatri and Sahu, 2011).

In this study, total phenols, tannins, and other phytochemicals were quantitatively studied, and the screening of phytochemicals was done qualitatively. Since ancient times, medicinal plants have been valued for their wide range of pharmacological effects, which are believed to be due to secondary plant metabolites such alkaloids, flavanoids, glycosides, tannins, steroids, etc. Certain plants are a significant source of naturally occurring antioxidants, which have been demonstrated to mitigate the risk and advancement of specific acute and chronic illnesses like cancer, heart disease, and stroke by scavenging free radicals, which are linked to the etiology of numerous diseases (Kavitha and Indira, 2016).

In the present study, three plants such as *Piper nigrum* L., *Citrus maxima* (Burm.f.) Merr., *Phyllanthus emblica* L. underwent qualitative and quantitative phytochemical investigation. The outcomes demonstrated that these plants contain significant amount of active substances. The

presence or absence of phytochemical in a sample is ascertained by the test used for phytochemical analysis. For more precise results, two or more distinct tests were performed. Out of three plant extracts, *Phyllanthus emblica* L. show more positive results for the phytochemical analysis.

It is feasible to isolate antioxidant compounds from plants by extracting them using various solvents; the type of extracting solvent used will determine this. Antioxidant's radical scavenging activity can be measured with ease, speed, and reliability using the DPPH assay. When the extract was compared to the standard antioxidant ascorbic acid, it demonstrated high DPPH radical scavenging activity. *Phyllanthus emblica* L. leaf extracts showed strong antioxidant activity. It was discovered that the DPPH scavenging action was dosage dependant (Shanmugapriya *et al.*, 2012).

Antioxidants were commonly identified in the DPPH test by their IC_{50} value, which stands for the Inhibition Concentration of Sample necessary to scavenge 50% of DPPH radicals. The dose response curve was plotted using concentration and % inhibition, and the findings were derived using linear regression analysis (Gayatri and Sahu, 2011).

The body's defense against damage from reactive oxygen species (ROS) is mostly dependent on antioxidants. Together with the respiratory chain's sporadic leaking, other metabolic processes that occur *in vivo* also generate these free radicals, also known as reactive oxygen species. The principal offenders in lipid peroxidation are these free radicals. There have been reports of plants with high antioxidant content that include phenolics (Shanmugapriya *et al*., 2012).

The oxidized form of DPPH, which has an absorbance of approximately 515–520 nm, is a stable free radical molecule. The DPPH assay is a rather quick and effective way to assess the scavenging activity of free radicals. To create a stable diamagnetic molecule, DPPH has the capacity to take an electron or a hydrogen radical. A shift in hue from purple to yellow signifies a reduction in the DPPH radical's absorption. This illustrates how antioxidants in mixed solutions interact with free radicals. In the current investigation, the antioxidant activity of the extracts that have the ability to inhibit free radicals was determined by measuring the percentage of inhibition.

The concentration of the sample needed to inhibit 50% of the radical was estimated using the IC_{50} value. The antioxidant activity of the samples increases with decreasing IC_{50} value. It is reported that extracts with IC_{50} values between 50 and 100 mg/ml is considered to exhibit intermediate antioxidant activity. Conversely, extracts having an IC_{50} value in the range of 10 to 50 mg/ml are considered to have strong antioxidant activity (Nurul *et al*., 2017).

Conclusion

Phyllanthus emblica L., *Citrus maxima* (Burm.f.) Merr., and *Piper nigrum* L. were found to possess rich source of valuable medical compounds. Various studies have confirmed that the identified phytochemicals in the selected leaf extracts are bioactive. The results revealed the presence of medically important constituents in the selected plants.

References

Ajmad K, Nain Taara B, Wajhat U, Iqbal N, Hamid AK, Khudija G, Muhammad A, Maha J, Noman K, Kainat SJ and Sidra F. (2023) Evaluation of antibacterial activity of *Piper nigrum* and *Cuminum* extracts on bacterial isolates from throat infection. J Population Therapeut Clin Pharmacol. 30(19):1412-1422.

Cameron, G.R, Milton RF and Allan JW, (1943) Measurement of flavonoids in plant sample. Lancet 2: 179.

Flama M, Shilpa SS, Ranjitha K, Shetty VV, Shetty PD, Patil P and Suchetha Kumari N. (2022) Phytochemical profiling, total flavanoid, total phenolic content and in-vitro antioxidant evaluation of *Citrus maxima* extract. Biomedicine 42(5): 912-919.

Ganesh P, Suresh Kumar R and Saranraj P. (2014) Phytochemical analysis and antibacterial activity of Pepper (*Piper nigrum* L.) against some human

pathogens. Central European J Exp Biol. 3(2): 36-41.

Gayatri N and Sahu RK. (2011) Phytochemical evaluation and antioxidant activity of *Piper cubeba* and *Piper nigrum*. J Appl Pharmaceut Sci. 1(8): 153-157.

Gurupriya S, Cathrine L and Ramesh J. (2017). Qualitative and quantitative phytochemical analysis of *Simarouba Glauca* leaf extract. Int J Res Appl Sci Engineer Technol. 5(11): 475-479.

Hangerman AE. (2002) Tannin Handbook. Miami University, Oxford, OH.

Hiai S, Oura H and Nakajima T. (1976) Color reaction of some sapogenins and saponins with vanillin and sulfuric acid. Planta Med. 29(2): 116-122

Junaid RS and Patil MK. (2020) Qualitative tests for preliminary phytochemical screening: An overview. Int J Chem Stud. 8(2): 603-608.

Kavitha S, Rameshkannan MV and Mani P. (2018) Analysis of antioxidant and antidiabetic activity of *Piper nigrum* leaf extract by in vitro assay. IOSR J Pharma Biol Sci. 13(2): 53-56.

Kenneth R, Chunpeng W, Amber T, Navindra PS and Hang M. (2018) Phenolic compounds isolated and identified from amla (*Phyllanthus emblica*) juice powder and their antioxidant and neuroprotective activities. Natural Prod Communic. 13(10): 1309-1311.

Larson RA. (1988) The antioxidants of higher plants. Phytochemistry 27: 969-978.

Malik CP and Singh MB. (1980) Estimation of polyphenols. In: Plant enzymology and histoenzymology, (eds). Malik C.P. and Singh M.B., Kalyani Publishers, New Delhi, pp. 286.

Muhammad A, Shinkafi SA, Farouk SNF. (2018) Phytochemistry and antibacterial activity of black pepper (*Piper nigrum*) seeds extracts on some food borne pathogens. Int J Pharmceu Sci Chem. 2018(1): 1-8.

Nurul J, Dewi H, Sylviana RH, Byan AA, Rizka YR and Wiwi W. (2017) Antioxidant activities of different solvent extracts of *Piper retrofractum* Vahl. using DPPH assay. AIP Conferences Proceedings. https://doi.org/10.1063/1.4985410

Shanmugapriya K, Saravana PS, Harsha P, Peer Mohammed S and Binnie W. (2012) Antioxidant potential of pepper (*Piper nigrum* Linn.) leaves and its antimicrobial potential against some pathogenic microbes. Indian J Natural Prod Resources 3(4): 570-577.

Showmiya K and Ananthi T. (2018) Phytochemical screening and FTIR analysis of *Citrus maxima* Linn. leaves. Int J Pharma Res Hlth Sci. 6(3): 2565-2569.

Evans WC. (2009) Trease and Evan's Pharmacognosy 16th edn., Saunders Ltd.., pp. 2075.

Yadav RNS and Agarwala M. (2011) Phytochemical analysis of some medicinal plants. J Phytology 3(12): 10-14.