



Plant Archives

Journal homepage: <http://www.plantarchives.org>
DOI Url : <https://doi.org/10.51470/PLANTARCHIVES.2023.v23.no1.048>

EVALUATION OF PHYTOCHEMICAL PROFILE, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF MAHONIA LESCHENAULTII (WALLICH EX WIGHT & ARNOTT) TAKEDA

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(Date of Receiving : 06-01-2023; Date of Acceptance : 12-03-2023)

ABSTRACT

The present study aims to determine the chemical composition of *Mahonia leschenaultii* (Wallich ex Wight & Arnott) Takeda collected in Ooty region (Nilgiris), have a phytochemical profile, anti-bacterial and to evaluate its antioxidant activity. The phytochemical screening helped us to highlight the presence of carbohydrates, proteins and amino acids, alkaloid, saponins, phenolic compounds, tannins, glycosides, flavonol glycosides and phytosterol. It showed that the ethanolic extract was very rich in total phenols (283.33 mg GAE/100 g), tannin (261.11 mg GAE/100 g) and flavonoid (56.51 mg RE/100 g). Moreover, ethanol extract exhibited good antibacterial activity against *Klebsiella pneumonia* (21.33 mm), *Staphylococcus aureus* (20.29 mm) and the ethyl acetate extract of fruit was also found as an antibacterial activity against *Escherichia coli* (23.23 mm), *Salmonella typhimurium* (22.27 mm) *Bacillus subtilis* (22.63 mm), at 20 mg/ml concentration. Ethyl acetate extract of fruit responded well against DPPH (21.03 µg/L), ABTS (62013.89 µM TE/g extracts), phosphomolybdenum (442.87 mg AAE/g extract) and superoxide (35.15 %). These results suggested that *M. leschenaultii* fruit extract, especially ethanol and ethyl acetate extract, is not only an important source of antioxidants, which possess a high potential source of antibacterial components.

Keywords : Anti-bacterial, Antioxidant, Anti-inflammatory, *Mahonia leschenaultii*

Introduction

Traditional medical practises frequently make use of medicinal plants because of their wide range of pharmacological effects and low toxicity in living organisms (Newman *et al.*, 2003; Damanhour and Ahamed, 2015). Focusing on drug discovery and development, researchers have studied medicinal plants and natural products for centuries (Rajedadram *et al.*, 2021). It is impossible to find new medications without the bioactive ingredients. Nearly two-thirds of all pharmaceuticals currently on the market can trace their roots back to nature, according to some estimates (Douglas Hanahan and Robert, 2000). Several, infectious diseases, including tuberculosis, diabetes, jaundice, hypertension, mental disorders, cancer, and AIDS, have been said to be cured with plant-based medications (Khan *et al.*, 2019).

There are a wide variety of biological effects attributed to antioxidants, including those that are anti-allergic, anti-atherogenic, anti-inflammatory, hepato-protective, antimicrobial, antiviral, antibacterial, anticarcinogenic, antithrombotic, cardioprotective, and vasodilatory (Ksouri *et al.*, 2007; Sumbul *et al.*, 2011). Diseases associated with oxidative stress can be avoided through the consumption of antioxidants derived from plants. The term "oxidative stress" refers to a condition when there is an excess of reactive oxygen species (ROS) compared to the body's natural antioxidant defences (Peluso *et al.*, 2012). Proteins, lipids, and nucleic acids are particularly susceptible to oxidative

stress, which is a major contributor to the development of many diseases, including cancer, atherosclerosis, diabetes, cardiovascular disease, age-related diseases, and inflammatory diseases (Islam *et al.*, 2015; Katerji *et al.*, 2019). Plant secondary metabolites, attributed to their antioxidant qualities and redox properties, play a crucial role in preventing further oxidative damage by quelling free radicals, consuming excess oxygen, or breaking down peroxides (Sumbul *et al.*, 2011).

Numerous plant-based medications with antioxidant, antibacterial, and therapeutic properties have been discovered around the world. Because bacteria can become resistant to antibiotics, researchers are constantly on the lookout for new therapeutic options. Antibiotic resistance can be reduced in a new way thanks to research into plant-based antimicrobials (Anowi *et al.*, 2012 and Alves *et al.*, 2013). The introduction of antibiotics treating microbial infections has been a breakthrough in contemporary medicine because of the significant impact it has had on lowering death and morbidity rates due to infectious diseases (Piddock, 2012). Certain minerals and enzymes, known as antioxidants or free radical scavengers, have been linked to a reduced risk of developing and a slowed progression of chronic diseases like cancer, cardiovascular disease, diabetes, and others. Antioxidants reduce the harmful effects of free radicals by facilitating their interaction with other molecules in a controlled chain reaction prior to any damage being done to essential components. Glutamine is one of the most well-known

antioxidants alongside phenolics, flavanoids, vitamins (E and C), and a plethora of minerals (Cu, Mn, Zn, Se, and Fe) (Abhishek *et al.*, 2013). It is well known that the widespread use of synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) in the food industry is linked to numerous health problems, including liver damage and carcinogenicity.

Species of *Mahonia* belong to the berberidaceae family of plants. Over 70 distinct *Mahonia* species can be discovered in every continent. Because of the bioactive compounds they contain, they may have medical use. The antibacterial, antifungal, anticancer, and anti-inflammatory properties of *Mahonia* spp. leaves, roots, stems, and barks have all been the subject of much research, and this is true even when the plants are not being used to treat skin diseases (Bournine *et al.*, 2017). *M. leschenaultii* is used as a medicine by various groups of people, especially the Toda of the Nilgiris. Those of the Toda tribe who live in India's Nilgiri region use a paste derived from the stem bark of the *M. leschenaultii* plant to alleviate postpartum ailments such as fever, a cold, and jaundice (Iqbal *et al.*, 2017). The purpose of this study was to examine the antibacterial and antioxidant properties of *M. leschenaultii* because of its promising medical applications.

Materials and Methods

Collection and Identification of Plant Material

Mahonia leschenaultii was obtained in its natural habitat in the Nilgiris district of Tamil Nadu, India, in the month of July 2022. Botanical study of India, Southern Circle, Coimbatore, and Tamil Nadu validated the plant's taxonomic identity. To remove any dirt or dust from the surface, we ran the fresh plant materials under running water and let them air dry in the shade. After that, a blender was used to grind them both into a fine powder that could be employed in experiments.

Preparation of Plant Extracts

The fruit powder was divided among many thimbles, and then extracted using a soxhlet apparatus with a series of organic solvents of increasing polarity. These solvents included petroleum ether, chloroform, ethyl acetate, and ethanol. After each round of solvent extraction, the thimble was allowed to air dry. After 24 hours of maceration in hot water with constant stirring, the extract water was filtered. After being extracted with various solvents, the concentrates were condensed using a rotary vacuum evaporator, air dried, and then put through their paces in further tests.

Phytochemical Screening

Mahonia leschenaultia fruit were experimented by using the standard method for determining phytochemicals (such as quinones, carbohydrates, alkaloids, reducing sugar, polyphenols, phenols, flavonoids, resins, glycoside, phlobatannins, xanthoproteins, triterpenoids, coumarins, cardial glycoside, cholesterol, etc.) for a preliminary qualitative phytochemical analysis (Raaman, 2006).

Quantification assays

Phenolic content

The Folin-Ciocalteu technique was used to calculate the total phenolic content of the flower extracts (Singleton and Rossi, 1965). Briefly, 2.0 mL of folin-ciocalteu reagent was

added to 100 μ L of sample extract (10 times pre-diluted). After waiting 5 minutes at room temperature, 1.6 mL of (2.5%, w/v) sodium carbonate solution was added, and the mixture was stirred thoroughly before being left to incubate for another 60 minutes. A UV-visible spectrophotometer (Thermo Scientific, Genesys 50, USA) was then used to determine absorbance at 765 nm. By employing a known concentration of gallic acid, a calibration curve of the proper shape was created. Gallic acid equivalents (GAE) per gramme of material were used to express all results.

Tannins content

Total phenolics includes tannin and non-tannin phenolic compounds. Total tannins were calculated by subtracting total phenolics from total phenolics (which included tannins) (including those that did not contribute to tannins). Each plant sample was incubated with 500 μ L, 100 mg of polyvinyl pyrrolidone (PVPP), and 500 μ L of distilled water in a 2 mL eppendorf tube for 4 hours at 4° C to determine the total non-tannin phenolics (Makkar, 2003). Following incubation, the Eppendorf tubes were centrifuged at 4° C for 10 minutes at 4000 rpm. The PVPP and any accompanying tannins would have been removed in the precipitation process, leaving only the non-tannin phenolics in the supernatant. We isolated the non-tannin phenolics by following the same procedure for quantifying total phenolics after collecting the supernatant. Each analysis was performed three times, and the results were expressed as Gallic acid equivalents. Combining these two methods, we were able to calculate the following tannin concentrations for each plant sampled: Tannins = Total phenolics - Non tannin phenolics

Flavonoid content

The total amount of flavonoids in the sample extracts was calculated using the aluminium chloride method reported in the study by Liu *et al* (2008). In summary, 2.5 mL of distilled water and sodium nitrite solution (5%, w/v, 150 mL) were added to 500 mL of the sample extract solution. After keeping this mixture at room temperature for 5 minutes, 300 mL of aluminium chloride (10%, w/v) was added and it was incubated for another 6 minutes. After that, 550 mL of distilled water were added, followed by 1 mL of sodium hydroxide (1 M). The absorbance of the solution was measured instantly at 510 nm using a UV-visible spectrophotometer after a thorough mixing (Thermo Scientific, Genesys 50, USA). The total flavonoid concentration was reported as Rutin equivalents (RE) per 100 g of dry sample.

Antibacterial activity

Bacterial strains

The Department of Biotechnology at Bharathiar University in Coimbatore provided the five strains of dangerous bacteria used in this study. The bacterial strains used were *Klebsiella pneumonia* (MTCC10309), *Escherichia coli* (MTCC405), *Bacillus subtilis* MTCC2057, *Salmonella typhimurium* (MTCC3224) and *Staphylococcus aureus* (MTCC9760).

Disk Diffusion Method

The disk diffusion method is employed to appraise antimicrobial activity of every plant extract. The plant extract residues (20 mg) were dissolved in 1 ml of DMSO. Muller Hinton agar was then prepared under sterile condition. The

organisms that were isolated was inoculated in the Nutrient broth and were incubated overnight. Then, swabs were used to lawn the organisms to the Muller Hinton agar plates and then, sterile discs were placed. The plant extract solution of 30 μL were poured into the sterile discs. The plates are incubated at 37°C overnight (Tendencia, 2004).

Antioxidant Activity

DPPH radical scavenging assay

Using the stable radical DPPH, the antioxidant activity of the extracts was quantified in terms of their hydrogen-donating or radical-scavenging capacity, as described by Gursoy *et al.* (2009). Extracts were extracted in volumes ranging from 20 to 100 μL , and the final volume was brought up to 100 μL using methanol. All of the aliquots of samples and standards (BHT and Rutin) were combined and vigorously agitated before a methanolic solution of DPPH (0.004%) was added. We made a Negative control by mixing 100 μL of methanol into 3 mL of methanolic DPPH solution. The tubes were left at 27 degrees for 30 minutes. At 517 nm, absorbance readings were made against a methanol blank to differentiate between test samples and the control. For each sample, we calculated its IC₅₀ value, or the concentration needed to inhibit 50% of DPPH concentration, to reflect its radical scavenging activity.

ABTS radical cation scavenging activity

The ABTS radical cation scavenging assay, following the protocol developed by Re *et al.* (1999). Was used to quantify the overall antioxidant activity. The ABTS radical cation was synthesised by incubating a 2.4 mM potassium persulfate aqueous solution with ABTS (stable radical) for 12-16 hours at room temperature in the dark. We first diluted the ABTS solution in ethanol (1:89 v/v) to an absorbance of 0.700 ± 0.02 at 734 nm before performing the test. Roughly 30 μL of sample solution and 10 μL of Trolox (final concentration 0-15 M) in ethanol were mixed with about 1 mL of diluted ABTS solution. One millilitre of diluted ABTS solution and thirty microliters of ethanol were used as a negative control in a test tube. We thoroughly vortexed all the test tubes and incubated them for 30 minutes at room temperature. After incubation, the samples' and the standards' (BHT and Rutin's) absorbances were measured at 734 nm against the ethanol blank. The results were reported in micromoles per gramme of sample extracts, which is the same as the concentration of Trolox needed to produce the same level of antioxidant activity.

Superoxide radical scavenging activity

The ability of the extracts to scavenge the superoxide radicals produced by the riboflavin-light-NBT system was used as the basis for the assay (Beauchamp and Fridovich, 1971). Add 100 μL of sample solution, BHT, and rutin to 3 mL of reaction mixture comprising 50 mM sodium phosphate buffer (pH-7.6), 20 g riboflavin, 12 mM EDTA, and 0.1 mg NBT. After exposing the reaction mixture with samples to light for 90 seconds, the reaction began. As a comparison, we compared the illuminating reaction mixture without sample. The absorbance was compared to a blank at 590 nm immediately after illumination (unilluminated reaction mixture without sample). The impact of scavenging on superoxide anion formation was determined by using the formula:

$$\text{Scavenging activity (\%)} = [(\text{Control OD} - \text{Sample OD}) / \text{Control OD}] \times 100$$

Phosphomolybdenum assay

Using a green phosphomolybdenum complex assay developed by Prieto *et al.* (1999), we were able to calculate the antioxidant activity of the sample. A blank of around 300 μL of methanol in a test tube was used. A series of test tubes were filled to a total volume of 300 μL with methanol and then divided into triplicates, each containing 100 μL of sample (1 mg/mL of respective organic solvents) and standards (BHT and rutin). Each test tube had 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) added to it and vortexed to combine the ingredients. After covering the test tube openings with foil, the tubes were heated in an incubator at 95 degrees Celsius for 90 minutes. Samples were allowed to cool to room temperature before being tested for absorbance at 695 nm against a reagent blank. All results were reported in terms of ascorbic acid equivalents (AAE) per milligrams of extract, with ascorbic acid serving as the reference standard.

Results and Discussion

Qualitative Phytochemical Screening

The results of a qualitative phytochemical screen performed on *M. leschenaultii* for its principal main and secondary phytochemicals are presented in Table 1. Carbohydrates, proteins, and amino acids were all found in all the extracts, as expected given the primary metabolites.

In addition to the primary metabolites, all samples contained secondary metabolites like alkaloids, saponins, phenolic compounds, tannins, glycosides, flavonol glycosides, cardiac glycosides, phytosterol, gums, and mucilage. Intense coloration suggests a high concentration of secondary metabolites (shown by a +++ sign), whereas a lack of coloration (represented by a - sign) suggests the absence of the corresponding chemical component. Cardiac glycosides, as well as gums and mucilage, were missing from the samples.

Antibacterial activity of plants extract

Antibacterial activity of *M. leschenaultii* fruit different extracts of chloroform, ethyl acetate, ethanol and hot water extract were checked against five human bacterial pathogens. The results of antibacterial activity of different extracts are presented in Table 2; Plate 1. Generally, the ethyl acetate and ethanol extract of fruit sample showed significant zone of inhibition *Klebsiella pneumonia* (21.33 mm), *Escherichia coli* (23.23 mm) and *Salmonella typhimurium* (22.27 mm), *Bacillus subtilis* (22.63 mm), *Staphylococcus aureus* (20.29 mm) at a concentration of 20 mg/mL. The widest zone of inhibition was observed in the ethanol extract of *M. leschenaultii* fruit extract while the value dropped constantly for ethanol, hot water, and chloroform. The virulent strains of *E. coli* cause gastroenteritis, urinary tract infections and neonatal meningitis. In rare cases it leads to haemolytic uremic syndrome, peritonitis, mastitis, septicemia, and gram-negative pneumonia (Todar, 2012). Most of the food borne illness are caused by *B. cereus* which causes nausea, vomiting and diarrhea (Kotiranta, 2000). The results revealed that fruit extract showed promising antibacterial activity. *Escherichia coli* is most stopped by a *M. leschenaultii* fruit extract made of ethyl acetate.

Quantification assays

Quantification of total phenolics, tannin and flavonoid

Tabulated in Table 3 are the results of an analysis of the total phenolics present in various extracts of *M. leschenaultii* fruit. The floral extract prepared by ethanol (283.33 mg GAE/100 g extract) had the highest phenolic content of all the sections tested. As opposed to other solvents, however, ethanol proved to be the most efficient at extracting phenolics. Generally, the amount and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolic molecules are primarily responsible for the free radical scavenging and antioxidant activity of phenolics (e.g., flavonoids, phenolic acids), while other parameters also play a role (Kahkonen *et al.*, 1999). Many plant species have had their total phenolics studied because of the potential contribution they may have to the species' antioxidant capability. Therefore, the greater concentration of phenolics in *M. leschenaultii* total extract is indicative of the species' superior antioxidant power. Table 2 displays the results of a study that determined the tannin content in solvent extracts of *M. leschenaultii* fruit. Ethanol extracts had a greater tannin concentration, with 261.11 mg GAE/100g extract on average. Ethanol's tannin-extraction efficacy was the highest compared to that of the other solvents. Recent research has shown that high molecular weight phenolics like tannins are more effective at reducing and scavenging free radicals. Tannins are used in many drugs because they make the body feel tight. They are used to treat haemorrhoids, diarrhoea, dysentery, leucorrhoea, and as a good throat medicine (Allport, 1970). Since leaf extracts had a low amount of tannins, there won't be any bad effects from tannins as anti-nutritional factors. Also, the tannins in the fruit of *M. leschenaultii* may make its extracts better at getting rid of free radicals. Table 3 shows the flavonoid content of the *M. leschenaultii* fruit, which was tested. It was found that ethanol extract (56.51 mg RE/g) had a good amount of flavonoids. It was also pointed out that ethanol could be used as a good solvent to get flavonoids out of *M. leschenaultii*. Flavonoids have been linked to several health benefits, such as preventing cancer, diabetes, ageing, and heart disease (Dixton *et al.*, 2005). So, measuring how much flavonoid is in a plant part is important because it can be linked to the plant's ability to fight cancer and get rid of free radicals. Since *M. leschenaultii* has large levels of flavonoids in its fruit, it is reasonable to presume that it has a potent free radical scavenging activity, which is achieved through the donation of an electron or hydrogen atom by the flavonoids to the free radicals.

In vitro antioxidant studies

DPPH scavenging activity

The DPPH assay is frequently utilised to evaluate the antioxidant activity of plant extracts and foods as free radical scavengers or hydrogen donors, and it is considered one of the most straightforward methods for determining antioxidant capacity (Siddhuraju and Becker, 2007). Scavenging actions against DPPH radicals by several *M. leschenaultii* fruit extracts are depicted in Figure 1. Extracts with a lower IC₅₀ value have more antioxidant activity. When compared to other solvent extracts, fruit extracts were among the most active of the many types of extracts tested. Ethanol extracts had substantial activity at a concentration of 21.03 µg/mL.

Plant extracts were also shown to have IC₅₀ values that were comparable to those of the standard natural antioxidant rutin and the synthetic antioxidant BHT. Extracts may include smaller compounds than bigger ones, which would explain why smaller molecules appear to have a stronger antioxidant capacity. This is because smaller molecules have easier access to the DPPH radical site (Yasodamma *et al.*, 2014).

ABTS cation radical and superoxide scavenging activity

The antioxidant capacity of hydrophilic and lipophilic substances in chosen test samples can be determined with the help of ABTS radicals, which are soluble in both aqueous and organic solvents and are unaffected by ionic strength (Sengul *et al.*, 2009; Zhou *et al.*, 2013). Table 4 shows the results of testing various *M. leschenaultii* extracts for their ability to scavenge ABTS cation radicals. Maximum radical scavenging activity (62013.89 µM TE/100g extract) was seen in the ethanol extract. Scavenging of ABTS⁺ by hot water extracts of all the extracts varied from 20833.33 to 62013.89 µM TE/100g extract.

Superoxide scavenging activities of *M. leschenaultii* fruit are revealed in Table 4. Ethanol extracts of the four plant components studied had the highest superoxide scavenging activity. The scavenging efficiency of these was highest (35.15%) in the ethanol extract. In humans, auto oxidative reactions can produce superoxide anion radical, a reactive oxygen species that is extremely damaging to cellular components. The ability of a plant extract to scavenge the superoxide radicals generated in a riboflavin-light NBT system is used in an assay measuring the link between reactive oxygen species (ROS) and oxidative stress. Hydrogen peroxide can be formed when the superoxide radical (which is created *in vivo*) reacts with oxygen (Halliwell and Gutteridge, 1986). Molecular oxygen can be converted into superoxides by oxidative enzymes and non-enzymatic reactions in the body. Scavenging superoxide radicals can either slow down the lipid peroxidation process or speed it up (Ilhami *et al.*, 2002). The active components in the plant extracts of *M. leschenaultii* may eliminate the radical by its reduction to attain the octant stage or through the creation of a water molecule, both of which are plausible mechanisms for scavenging superoxide anion radicals.

Phosphomolybdenum assay

Extracts' ability to decrease Mo (VI) to Mo (V) and then form a green phosphate /Mo (V) complex at an acidic pH can be determined with the help of the phosphomolybdenum test. With its ease of use and independence from other regularly used antioxidant assays, it was chosen to use this method to plant extracts rather than just vitamin E in seed quantification (Prieto *et al.*, 1999). Free radical scavenging activity comparable to that of natural antioxidant ascorbic acid correlates with the total antioxidant capacity measured for *M. leschenaultii* extracts. Ethanol extracts *M. leschenaultii* fruit confirmed that effectively very high reducing ability (442.87 mg AAE/g extract) however the petroleum ether extracts might show off the important extent of reducing ability (345.59 mg AAE/g extract) when compared to other extracts. The bioactive chemicals, especially the phenolics and flavonoids, found in the various portions may be responsible for the reduction of Molybdenum by the fruit extracts through the transfer of electrons or hydrogen ions (Fig. 2).

Table 1 : Phytochemical screening of *M. leschenaultii*

Phytochemicals	Fruits
Carbohydrates	+++
Proteins	+++
Amino acids	+++
Alkaloids	+++
Saponins	+
Phenolic compounds	+++
Tannins	++
Glycosides	++
Flavonol glycosides	++
Cardiac glycosides	-
Phytosterols	+++
Gums and mucilages	-

(+): Presence of chemical compound, (-): Absence of chemical compound; (+) < (++) < (+++): Based on the intensity of characteristic colour

Table 2 : Anti-bacterial activity of *M. leschenaultii* fruit extract

Extracts	Concentration (mg/mL)	Diameter of Zone of inhibition (mm)				
		Gram Negative			Gram Positive	
		<i>Klebsiella pneumonia</i>	<i>Escherichia coli</i>	<i>Salmonella typhimurium</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>
Negative control	-	-	-	-	-	-
Chloroform	20	14.16 ± 0.12 ^c	14.12 ± 0.12	15.29 ± 0.17	17.28 ± 0.22 ^b	15.22 ± 0.13
Ethyl acetate	20	20.03 ± 0.79 ^a	23.23 ± 0.49^b	22.27 ± 0.13^b	22.63 ± 0.47^a	19.34 ± 0.25 ^c
Ethanol	20	21.33 ± 0.23^a	22.31 ± 0.19 ^c	21.23 ± 0.20 ^c	22.3 ± 0.29 ^a	20.29 ± 0.22^b
Hot Water	20	16.23 ± 0.23 ^b	17.28 ± 0.19	17.22 ± 0.15	17.37 ± 0.23 ^b	15.17 ± 0.12
Positive control	15 mcg	21.26 ± 0.10 ^a	24.11 ± 0.17 ^a	23.69 ± 0.36 ^a	21.98 ± 0.71 ^a	22.58 ± 0.50 ^a

Table 3: Phenolic, Tannin and flavonoids content of *Mahonia leschenaultii* fruit

Extracts	Phenolic GAE/g extract	Tannin GAE/g extract	Flavonoids RE/100 g
Petroleum ether	21.92 ± 1.75	21.26 ± 1.62 ^c	8.48 ± 0.58
Chloroform	59.94 ± 1.33 ^d	55.56 ± 56 ^c	9.57 ± 1.96
Ethyl acetate	187 ± .87 ^c	161.87 ± 2.04 ^a	24.74 ± 1.12 ^c
Ethanol	283.33 ± 1.51^a	261.11 ± 1.82^a	56.51 ± 0.96^a
Hot Water	210.52 ± .87 ^b	194.54 ± 1.59 ^b	33.59 ± 1.63 ^b

Values are mean of triplicate determination (n=3) ± standard deviation, statistically significant at p < 0.05 where ^{a>b>c>d} in each column

Table 4 : ABTS scavenging activity and Superoxide radical of *M. leschenaultii* fruit

Sample	Extracts	ABTS scavenging Activity (µM TE/g extract)	Superoxide radical scavenging activity Percentage of Inhibition (%)
<i>M. leschenaultii</i>	Petroleum ether	20833.33 ± 890	8.92 ± 0.78
	Chloroform	43541.67 ± 925.85	6.32 ± 1.09
	Ethyl acetate	56909.72 ± 1170.81 ^c	12.40 ± 1.51
	Ethanol	62013.89 ± 2485.49^b	35.15 ± 1.31^b
	Hot Water	58472.22 ± 2398.89 ^c	17.35 ± 1.31 ^c
Standard	Rutin	96875 ± 208.33 ^a	74.7 ± 0.25 ^a
	BHT	97465.28 ± 318.23 ^a	74.2 ± 0.1 ^a

Values are mean of triplicate determination (n = 3) ± standard deviation, TE- Trolox Equivalents, statistically significant at p < 0.05 whereas ^{a>b>c>d} in each column.

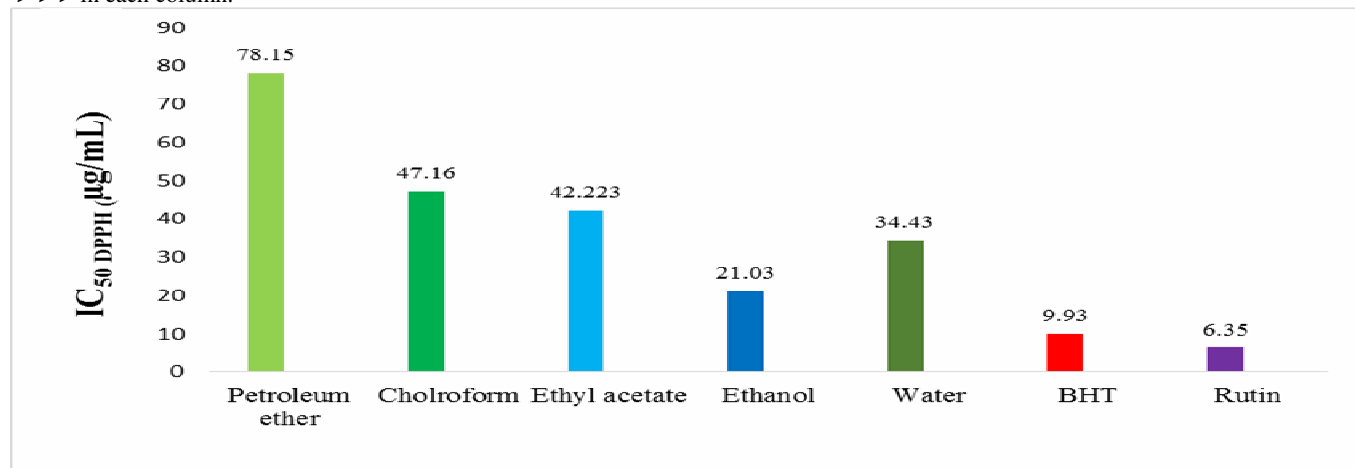


Fig. 1: DPPH scavenging activity of *M. leschenaultii*

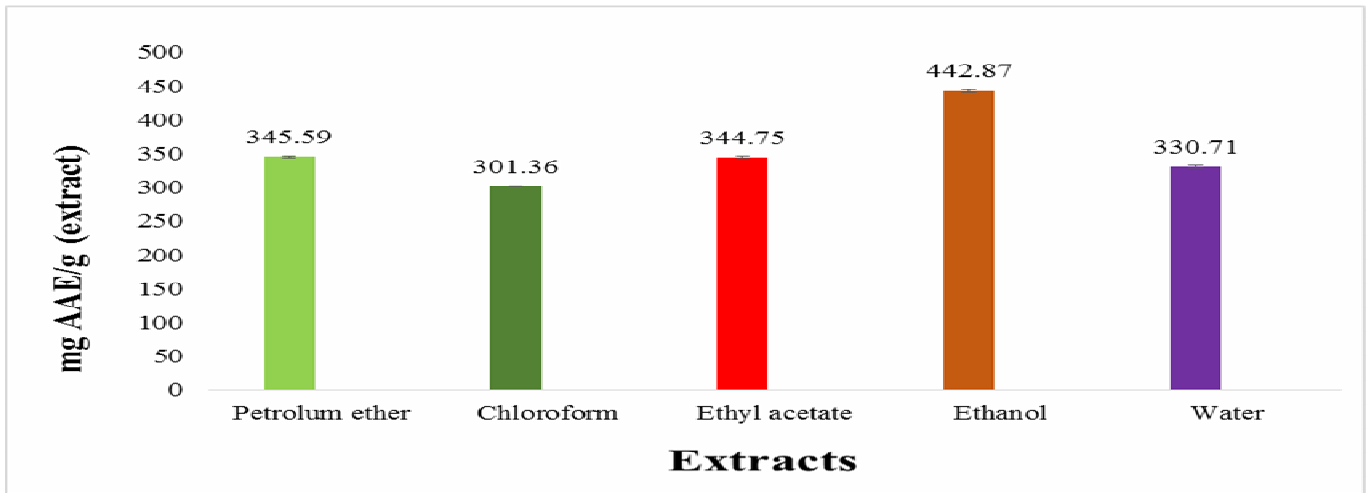
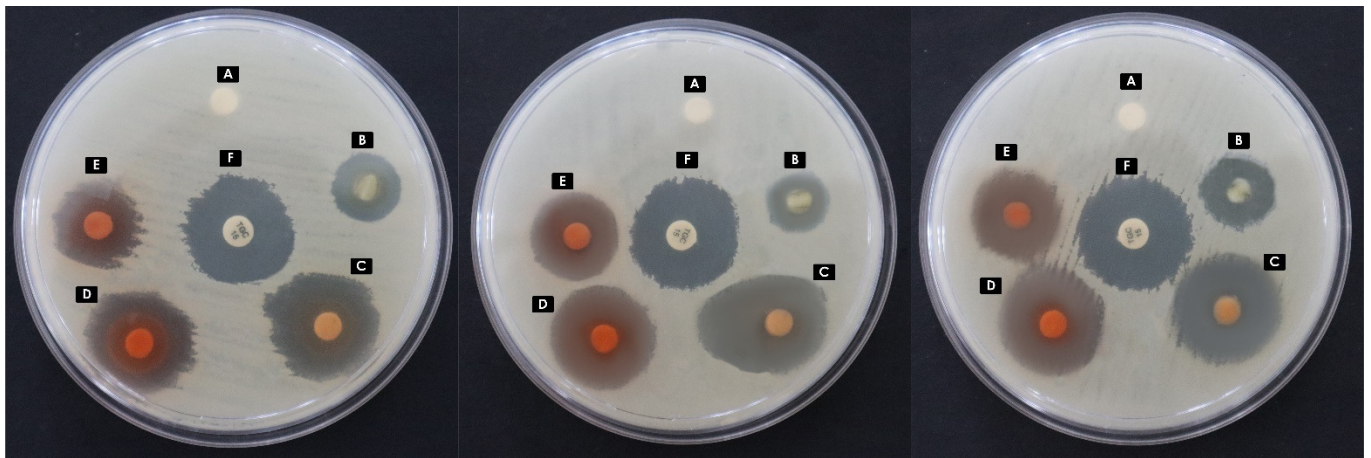


Fig. 2 : Phosphomolybdenum Assay of *Mahonia leschenaultii* fruit

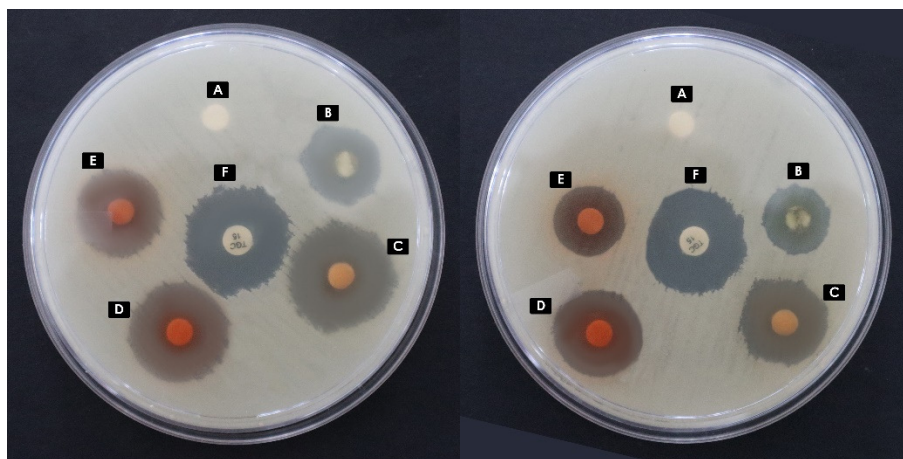
PLATE 1
Anti-bacterial activity of *M. leschenaultii* fruit Extracts



Klebsiella pneumonia

Escherichia coli

Salmonella typhimurium



Bacillus subtilis

Staphylococcus aureus

A: Negative Control (DMSO), B: Chloroform, C: Ethyl acetate, D: Ethanol, E: Hot water, F: Positive Control (Tigecycline).

References

- Abhishek, R.U.; Devihalli, C.M. and Manjunath, K. (2013). Antioxidant properties of some selected Indian medicinal plants: Their correlation with total phenolic contents. *International journal of Green Pharmacy*, 7(2): 117-120. DOI: 10.4103/0973-8258.116388
- Alam, M.S.; Damanhour, Z.A.; Ahmad, A.; Abidin, L.; Amir, M.; Aqil, M.; Khan, S.A. and Mujeeb, M. (2015). Development of response surface methodology for optimization of extraction parameters and quantitative estimation of embelin from *Embelia ribes* Burm by high performance liquid chromatography. *Pharmacogn Mag.* 11: S166-72. doi: 10.4103/0973-1296.157722
- Allport, G.W.; Vernon, P.E. and Lindzey, G. (1970). Study of Values (Revised 3rd ed.). Riverside Publishing.
- Anowi, C.F.; Onyegbule, A.F.; Gugu, T.H. and Utoh-Nedosa, U.A. (2012). Evaluation of Antimicrobial Properties of N-Hexane Extract of the Leaves of *Napoleoneae imperialis* Family Lecythiaceae. *Int J Pharm Sci Res*, 3(7): 2154-2158. DOI: [http://dx.doi.org/10.13040/IJPSR.0975-8232.3\(7\).2154-58](http://dx.doi.org/10.13040/IJPSR.0975-8232.3(7).2154-58)
- Beauchamp, C. and Fridovich, I. (1971) Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels. *Analytical Biochemistry*, 44: 276-287.
- Bournine, L.; Bensalem, S.; Fatmi, S.; Bedjou, F.; Mathieu, V.; Iguer-Ouada, M. and Duez, P. (2017). Evaluation of the cytotoxic and cytostatic activities of alkaloid extracts from different parts of *Peganum harmala* L. (Zygophyllaceae). *European Journal of Integrative Medicine*, 9: 91–96. doi:10.1016/j.eujim.2016.10.002
- Dixon, J.; Durrheim, K. and Tredoux, C. (2005). Beyond the Optimal Contact Strategy: A Reality Check for the Contact Hypothesis. *American Psychologist*, 60(7): 697–711. doi:10.1037/0003-066x.60.7.697
- Gursoy, N.; Sarikurkcu, C.; Cengiz, M. and Solak, M.H. (2009). Antioxidant activities, metal contents, total phenolics and flavonoids of seven *Morchella* species. *Food and Chemical Toxicology*, 47(9): 2381–2388.
- Halliwell, B. and Gutteridge, J.M.C. (1986). Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. *Archives of Biochemistry and Biophysics*, 246(2): 501–514. doi:10.1016/0003-9861(86)90305-x
- Hanahan, D. and Weinberg, R.A. (2000). The hallmarks of cancer. *Cell*. 100(1): 57-70. doi: 10.1016/s0092-8674(00)81683-9
- Ilhami, N. and Khalid, M. (2002). Testosterone Dependence of Salt-Induced Hypertension in Sabra Rats and Role of Renal alpha 2-Adrenoceptor Subtypes. *Journal of Pharmacology and Experimental Therapeutics*, 300(1): 43–49.
- Iqbal, J.; Abbasi, B.A.; Mahmood, T.; Kanwal, S.; Ali, B.; Shah, S.A. and Khalil, A.T. (2017). Plant-derived anticancer agents: A green anticancer approach. *Asian Pacific Journal of Tropical Biomedicine*, 7(12): 1129–1150. doi:10.1016/j.apjtb.2017.10.016
- Kahkonen, M.P.; Hopia, A.I.; Vuorela, H.J.; Rauha, J.-P.; Pihlaja, K.; Kujala, T.S. and Heinonen, M. (1999). Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. *Journal of Agricultural and Food Chemistry*, 47(10): 3954–3962. doi:10.1021/jf990146l
- Katerji, M.; Filippova, M. and Duerksen-Hughes, P. (2019). Approaches and Methods to Measure Oxidative Stress in Clinical Samples: Research Applications in the Cancer Field. *Oxidative Medicine and Cellular Longevity*, 1–29. doi:10.1155/2019/1279250
- Khalid, M. (2002). Testosterone Dependence of Salt-Induced Hypertension in Sabra Rats and Role of Renal alpha 2-Adrenoceptor Subtypes. *Journal of Pharmacology and Experimental Therapeutics*, 300(1): 43–49. doi:10.1124/jpet.300.1.43
- Khan, W.; Subhan, S.; Shams, D.F.; Afridi, S.G.; Ullah, R.; Shahat, A.A. and Alqahtani, A.S. (2019). Antioxidant Potential, Phytochemicals Composition, and Metal Contents of *Datura alba*. *BioMed Research International*, 1–8. doi:10.1155/2019/2403718
- Kotiranta, A.; Lounatmaa, K. and Haapasalo, M. (2000). Epidemiology and pathogenesis of *Bacillus cereus* infections. *Microbes and Infection*, 2(2): 189–198. doi:10.1016/s1286-4579(00)00269-0
- Ksouri, R.; Megdiche, W.; Debez, A.; Falleh, H.; Grignon, C. and Abdelly, C. (2007). Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. *Plant Physiology and Biochemistry*, 45(3-4): 244–249. doi:10.1016/j.plaphy.2007.02.001
- Liu, C.J.; Hsiung, P.C.; Chang, K.J.; Liu, Y.F.; Wang, K.C.; Hsiao, F.H.; Ng, S.M. and Chan, C. (2008). A study on the efficacy of body-mind-spirit group therapy for patients with breast cancer. *Journal of Clinical Nursing*, 17: 2539–2549.
- Makkar, H.P.S. (2003) Effects and Fate of Tannins in Ruminant Animals, Adaptation to Tannins, and Strategies to Overcome Detrimental Effects of Feeding Tannin-Rich Feeds. *Small Ruminant Research*, 49: 241-256.
- Mohd. Akhtar, Ahmad, M.A.; Sumbul, S. and Mohd, Asif. (2011). Role of phenolic compounds in peptic ulcer: An overview. *Journal of Pharmacy and Bioallied Sciences*, 3(3): 361. doi:10.4103/0975-7406.84437
- Newman, D.J.; Cragg, G.M. and Snader, K.M. (2003). Natural Products as Sources of New Drugs over the Period 1981–2002. *Journal of Natural Products*, 66(7): 1022–1037. doi:10.1021/np030096l
- Peluso, I.; Morabito, G.; Urban, L.; Ioannone, F. and Serafi, M. (2012). Oxidative Stress in Atherosclerosis Development: The Central Role of LDL and Oxidative Burst. *Endocrine, Metabolic & Immune Disorders-Drug Targets*, 12(4): 351–360. doi:10.2174/187153012803832602
- Piddock, L.J. (2012). The crisis of no new antibiotics—what is the way forward? *The Lancet Infectious Diseases*, 12(3): 249–253. doi:10.1016/s1473-3099(11)70316-4
- Prieto, P.; Pineda, M. and Aguilar, M. (1999). Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Analytical Biochemistry*, 269(2): 337–341. doi:10.1006/abio.1999.4019
- Raaman N, Phytochemical techniques. New India Publishing Agency. Jai Bharat Printing, Press. New Delhi, 19-22, 2006.
- Rahman, M.M.; Islam, M.B.; Biswas, M. *et al.* (2015). *In vitro* antioxidant and free radical scavenging activity of

- different parts of *Tabebuia pallida* growing in Bangladesh. *BMC Res Notes*. 8: 621 <https://doi.org/10.1186/s13104-015-1618-6>
- Rajedadram, A.; Pin, K.Y.; Ling, S.K.; Yan, S.W. and Looi, M.L. (2021). Hydroxychavicol, a polyphenol from *Piper betle* leaf extract, induces cell cycle arrest and apoptosis in TP53-resistant HT-29 colon cancer cells. *J Zhejiang Univ Sci B*. 22(2): 112-122. doi: 10.1631/jzus.B2000446.
- Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M. and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9-10): 1231–1237.
- Sengul, M.; Yildiz, H.; Gungor, N.; Cetin, B.; Eser, Z. and Ercisli, S. (2009). Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. *Pak J Pharm Sci*. 22(1): 102-6.
- Siddhuraju, P. and Becker, K. (2007). The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) Seed extracts. *Food Chemistry*, 101(1): 10–19. doi:10.1016/j.foodchem.2006.01.00
- Singleton, V. and Rossi, J. (1965) Colorimetry of Total Phenolic Compounds with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, 16, 144-158.
- Soares, M.O.; Alves, R.C.; Pires, P.C.; Oliveira, M.B.P.P. and Vinha, A.F. (2013). Angolan *Cymbopogon citratus* used for therapeutic benefits: Nutritional composition and influence of solvents in phytochemicals content and antioxidant activity of leaf extracts. *Food and Chemical Toxicology*, 60: 413–418. doi:10.1016/j.fct.2013.07.064
- Tendencia, E.A. (2004). Disk diffusion method. In *Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment*: Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center. 2004; 13-29.
- Todar, K. (2012). Molecular Characterization of Extended Spectrum β -Lactamase Genes in Clinical *E. coli* Isolates. *Journal of Biomedical Science and Engineering*, 7: 5.
- Yasodamma, N.; Chaithra, D. and Alekhya, Chennuri. (2013). Phytochemical screening of *Curcuma neilgherrensis* WT. An endemic medicinal plant from Seshachalam hills (A.P) India. *International Journal of Pharma and Bio Sciences*. 6: 409-412.
- Zhou, Z.Q.; Li, L.P.; Xu, Z.H.; Zhang, Q.Q. Li.; S.C. and Shi, S.S. (2013). Risk assessment of water inrush in karst tunnels based on attribute synthetic evaluation system. *Tunnelling and underground space technology*, 38: 50-58.