



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 2052-2058
Received: 08-05-2017
Accepted: 10-06-2017

S Irudaya Monisha
Research Scholar, Department of
Chemistry, Holy Cross College
(Autonomous), Trichy-1, Tamil
Nadu, India

G Dayana Jeya Leela
Research Scholar, Department of
Chemistry, Holy Cross College
(Autonomous), Trichy-1, Tamil
Nadu, India

A Anitha Immaculate,
Department of Chemistry,
Faculty at Botho University,
Botswana, South Africa.

J Rosaline Vimala,
Assistant Professor, Department
of Chemistry, Holy Cross College
(Autonomous), Trichy-1, Tamil
Nadu, India

Comparative studies on yield and the phytochemical appraisal (Quality and Quantity) of *Manilkara hexandra* (Roxb) Dubarb using leaf, stem, and bark

S Irudaya Monisha, G Dayana Jeya Leela, A Anitha Immaculate and J Rosaline Vimala

Abstract

The indigenous plant of *Manilkara hexandra* belongs to Sapotaceae family. It is also called in tamil "Kanuppalai". The sacred plant is widely observed in the religious temple of South India because of it consists of latex. The investigation is carried on phytochemical screening analysis, quantitative and yield percentage of leaf, stem, and bark of *Manilkara hexandra*. The dried powder was screened with petroleum ether, hexane, chloroform, ethyl acetate, ethanol, methanol and double distilled water by cold extraction method. The secondary metabolites were estimated with standard procedures. The yield percentage was calculated. The qualitative report shows the presence of phytochemicals. The high quantity of alkaloids ($0.153.8 \pm 45.8$ mg/g), saponins (211 ± 70.40 mg/g) were detected in stem, flavonoids (144 ± 3.16 mg/g) in leaf, terpenoids (34.0 ± 4.1 mg/g) in bark. The phenol (9.49 ± 0.0002 mg/g) and the tannin (79.03 ± 0.019 mg/g) present in the bark was examined by using standard gallic acid and tannic acid respectively. The yield reveals that the amount of stem (27.64 g in %) is rich in ethanol. From the consecutive results, concluded that the rich herbal compounds are utilized for future research on innovative drug delivery system.

Keywords: *Manilkara hexandra*, Yield, Qualitative, Quantitative analysis.

1. Introduction

A natural product plays an important role in the field of new drugs research and development, because of their low toxicity, easy availability and cost-effective [1]. According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs [2]. *Manilkara hexandra* grows in natural wild conditions and mainly propagated through seeds. Its usage has been reported mostly in the traditional medicinal system of India. The leaf, stem, bark & fruit of the plant consists of various medicinal values such as astringent, refrigerant, aphrodisiac, alexipharmic, stomatic, anthelmintic. It is also used in menorrhagia, odontopathy, Fever, Colic, Dyspepsia, helminthiasis, burning sensation, vitiated conditions of pitta it retards, the fermentation process and loss of consciousness, hallucination, anorexia, and leprosy.

Traditionally it issued in medicinal herbal drugs to cure various diseases such as jaundice, ulitis, odontopathy, fever, colic dyspepsia, helminthiasis, hyper dyspepsia and burning sensation [3]. The latex of tree is applied on teeth and gums for toothaches. A survey from Jalgaon district of North Maharashtra shows that its fruits are used to relieve digestive disorder [4]. It purifies the blood and beneficial in swelling, abdominal colic, gout, rheumatism and toxicosis [5]. This drives the need to screen medicinal plants of *Manilkara hexandra* for novel bioactive compounds as plant-based drugs are biodegradable and safe. The phytochemical screening of plants is the need to develop the drugs. Enormous research groups have also reported such studies throughout the world [6-10]. Phytonutrients are vital role in health promotion and disease prevention [11-12]. This Phytochemical Screening, quantitative report and yield percentage aimed that the present bioactive phytochemicals are utilized in their future perspectives of medicinal property and the isolation of active compounds from *Manilkara hexandra* for drug delivery system.

Correspondence
S Irudaya Monisha
Research Scholar, Department of
Chemistry, Holy Cross College
(Autonomous), Trichy-1, Tamil
Nadu, India

Kingdom	Plantae
Phylum	Tracheophyta
Subphylum	Euphyllophytina
Class	Magnoliopsida
Subclass	Magnoliidae
Order	Ericales
Super order	Asteranae
Family	Sapotaceae
Subfamily	Sapotoipeae
Tribe	Mimosopeae
Subtribe	Manikarinae
Genus	Manilkara
Subgenus	Nom
Species	Manilkara hexandra

The botanist position of the genus *Manilkara hexandra*. (Roxb) Dubarb^[13] as in Fig.1

Materials and Methods

The Collection of Plant Materials

The Leaf, Stem, and Bark of *Manilkara hexandra* are used in Ayurveda and traditional systems of medicine were collected from Jayakondam at Ariyalur District, Tamil Nadu in India. The plant was identified by Rev. Fr. S. John Britto, The Rapinet Herbarium, at St. Joseph College, Trichy. The voucher sample was preserved (Voucher No.0011). (Fig.2)



Fig. 2: *Manilkara hexandra*

Preparation of plant powder

The collection of the plant material (leaf, stem, and bark) was washed under running tap water to remove the surface pollutants. The materials were dried at room temperature under the shade for three weeks and size reduced manually using mortar.

Preparation of solvent extraction by cold percolation method

The powdered portion of leaf, stem, and bark (10g) were weighed in an analytical weighing balance. The plant powder was soaked in a dark brown bottle with 100 ml of solvents such as petroleum ether, hexane, chloroform, ethyl acetate, ethanol, methanol and double distilled water separately. These 21 bottles were kept in a dark room for 72 hours. After 3 days the extracts were filtered through the No-1. What manTm filter paper and the extracts were preserved in the refrigerator.

Yield percentage

The production of phytochemicals were yielded from leaf, stem, and bark of *Manilkara hexandra*. The preserved extracts were taken and evaporated by a water bath at 60°C to get dried crude extract. The differences between before and after the constant weight of the container were noted. The yield percentage of 21 solvent extracts were calculated using the formula^[14].

$$\% = (W2 - W1) / W0 \times 100$$

W2 - Weight of the crude extract

W1 - Weight of the container

W0 - Weight of the initial dried sample

Qualitative analysis

The identification of phytochemicals were screened by the standard procedures. The screening test of 21 solvent extracts such as petroleum ether, hexane, chloroform, ethyl acetate, ethanol, methanol and double distilled water were carried out for leaf, stem, and bark of *Manilkara hexandra*. These solvent extracts were exposed to the presence or absence of secondary metabolites like alkaloids, steroids, phenolic compounds, flavonoids, saponins, tannins and anthraquinones using standard procedure^[15-18]. Primary metabolites such as starch, quinones, oxalate^[19], Fixed oils and fats^[20] and gum and mucilages^[21].

Phytochemical Screening

The Phytochemical analysis were carried out by using petroleum ether, hexane, chloroform, ethyl acetate, ethanol, methanol and double distilled water extracts of leaf, stem, and bark of *Manilkara hexandra* plant in this study.

1. Detection of Alkaloids

The extract was dissolved individually in dil. HCl acid Solution was clarified by filtration.

a). Mayer's Test

The filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide) to the formation of a yellow color precipitate indicates the presence of alkaloids.

b). Wagner's Test

The filtrate was treated with Wagner's reagent to give the color of brown or reddish precipitate which indicates the presence of alkaloids.

c). Dragendroff's Test

The filtrate was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d). Hager's Test

The filtrate was treated with Hager's reagent (saturated picric acid solution) to the formation of the yellow colored precipitate indicates the presence of alkaloids.

2. Detection of Amino acid

Ninhydrin Test

The test solution is boiled with 0.2% solution of ninhydrin, would result in the formation of purple color suggesting the presence of free amino acids.

3. Detection of Proteins

Biuret Test

The extract was treated with NaOH and few drops of CuSO₄ Solution and which shows the formation of violet or pink color indicates the presence of proteins.

4. Detection of Flavonoids

a) Ferric Chloride Test

The filtered solution of the extract was treated with three drops of freshly prepared 1% Ferric Chloride and potassium ferrocyanide to the formation of bluish- green color shows the presence of phenolic compounds.

b) Alkaline Reagent Test

The Extract was treated with few drops of sodium hydroxide solution to the formation of intense yellow color, after the addition of dilute HCl acid gives colorless, which indicates the presence of flavonoids.

c) Lead Acetate Test

The Extract was treated with few drops of lead acetate solution. Formation of yellow color precipitate which indicates the presence of flavonoids.

5. Detection of Anthraquinones

Borntrager's test

The extract was shaken with 10 ml of benzene, filtered, and 5 ml of 10% ammonia solution added to the filtrate, formation of a pink, red, or violet color in the ammoniacal phase which indicates the presence of anthraquinones.

6. Detection of Steroids

a) Salkowski's Test

The extract was dissolved in 2 ml chloroform in a test tube. Con. H₂SO₄ acid was carefully added to the wall of the test tube to form a lower layer. A reddish brown color at the interface indicated the presence of a steroid ring (i.e., the aglycone portion of the glycoside).

b) Libermann Burchard's Test

The Extract was treated with chloroform and added the few drops of acetic anhydride, boiled and then cooled to add Con. H₂SO₄ acid to the formation of a brown ring at the junction, indicates the presence of phytosterols.

7. Detection of Terpenoids

The extract was added to 2 ml of acetic anhydride and Con. H₂SO₄ acid which appears of blue or green colored rings indicates the presence of terpenoids.

8. Detection of Tannins

a) Ferric Chloride Test

The extract was treated with ferric chloride solution to change the color into bluish-black which indicates the presence of tannin.

b) Lead Acetate Test

The extract was dissolved in water and 10% Lead acetate solution was added to get the appearance of yellow precipitate confirms the tannins.

c) Potassium dichromate Test

The extract was dissolved in water and then added potassium dichromate solution, to give a yellow color precipitate indicates the presence of tannins and phenolic compounds.

9. Detection of Saponins

Froth Test

The extract was added with distilled water to shake vigorously for 15 minutes after the form of froth layer (1 cm) indicates the presence of saponins.

10. Detection of Anthocyanins

The extract was added to 2 ml of 2N HCl and ammonia. Initial appearance of pink-red color turning into blue-violet indicates the presence of anthocyanins.

11. Detection of Coumarins

3 ml of 10% NaOH was added to the extract, formation of

yellow color indicates the presence of coumarins.

12. Detection of Emodins

2 ml of NH₄OH and 3 ml of Benzene was added to the extract. An appearance of red color indicates the presence of emodins.

13. Detection of Reducing Sugars

The Extracts was diluted in 5 ml distilled water and filtered. The filtrate is used to the following tests for carbohydrates.

a). Fehling's Test:

Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

b). Keller - Kilian test (for deoxy sugars in cardiac glycosides):

The methanol extract was obtained and the extract was dried. 50 mg of this was dissolved in 2 ml chloroform, H₂SO₄ was added to form a layer and the color at interphase was recorded. Brown ring at interphase is characteristic of deoxysugars in cardenolides.

14. Detection of fixed Oils and Fats

a) Spot test: A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oil.

b) Saponification test: A few drops of alcoholic KOH solution is added to the extract along with a drop of phenolphthalein. This mixture is heated for 2 h. by using water bath till the formation of soap or viscous in nature of alkali indicates the presence of fixed oils and fats.

15. Detection of Gum and Mucilages

The extract is dissolved in 10 ml of distilled water and 25 ml of absolute alcohol is added. It is stirred constantly to get the White or cloudy precipitate which indicates the presence of gums and mucilages.

16. Detection of glycosides

The extract was dissolved in 1 ml of water and then aqueous NaOH Solution was added. Formation of yellow color indicates the presence of glycosides.

17. Detection of Phlobatannins

Precipitate test:

The extract was boiled with 1ml of dil. HCl to get the red precipitate. This deposition is the proof of the presence of phlobatannins.

18. Detection of Quinones

A small amount of extract was treated with Con. HCl and observed for the formation of the yellow precipitate.

19. Detection of resins

The extract was treated with acetic anhydride and then added Con. H₂SO₄. Shows the color orange to yellow which indicates the presence of resins.

20. Detection of Xanthoproteins

The Extract was treated with Con. HNO₃ and added NH₃ solution to give the reddish orange precipitate which indicates the presence of xanthoproteins.

Quantitative analysis

The plant samples of leaf, stem, and bark of Manilkara hexandra were used in the following procedure to determine the amount of secondary metabolites such as, alkaloid, flavonoid, terpenoid, saponin, phenol and tannin. These estimated procedures were carried out for three times simultaneously.

Determination of Alkaloid

2g of the sample (leaf, stem, and bark) was taken in a 250ml beaker and 200ml of (10%) acetic acid in ethanol was added and covered and allowed to stand for 4hours. This was filtered and the extract was concentrated on a water bath to one – quarter of the original volume. The addition of concentrated ammonium hydroxide in the whole solution was allowed to settle down in the vessel. The settled portion was collected and washed with dilute NH₄OH and then filtered. This residue (alkaloid) was dried and weighed [22].

Determination of Flavonoid

2g of the sample (leaf, stem, and bark) was extracted with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered and transferred into a crucible. The crucible was weighed before and after evaporated to dryness over a water-bath to be cooled to get the constant weight of the flavonoid [23].

Determination of Terpenoids

2g of the sample (leaf, stem, and bark) were taken separately and soaked in 50 ml alcohol for 24 hours and then filtered. The filtrate was treated with 40 ml of petroleum ether for 2 hours. This filtrate was transferred into a crucible and evaporated to dryness over a water-bath and weighed to get a constant weight of terpenoid [24].

Determination of Saponin

2g of the sample (leaf, stem, and bark) was taken into a conical flask and 50ml of 20% aqueous ethanol was added. These samples were heated over a hot water bath for 4hours with constant stirring at about 55°C. This mixture was filtered and the residue was re-extracted with another 100ml of 20% ethanol. The collection of the extract was reduced to 40ml over the water bath at about 90°C and the concentrated extract (leaf, stem, and bark) was transferred into a separating funnel and 10ml of diethyl ether was added and shaken vigorously. The aqueous layer was collected while the ether layer was discarded. The purification process was repeated and the addition of n-butanol (30ml). The extract (leaf, stem, and bark) of n-butanol was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight of saponins [25].

Estimation of Phenol

1ml of the methanol diluted plant sample (leaf, stem, and bark) or Gallic acid standard phenolic compound was added to a 25 ml volumetric flask, containing 9 ml of distilled water. 1 ml of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na₂CO₃ solution was mixed in to the test sample solution was diluted to 25 ml distilled water and mixed thoroughly. The mixture was kept in the dark

for 90 min at 23°C, after which the absorbance was read at 750 nm. Total phenol content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution (20 to 80 µg/ml). The Total Phenolic content was expressed as milligrams of Gallic acid (GAE) equivalents per gram dried sample [26].

Estimation of Tannin

5 g of the plant sample (leaf, stem, and bark) were boiled with 400 ml of water for 1 hour, cool and makeup to 500 ml in a standard flask. 0.2ml of plant extracts containing 7.5 ml of water. Add 0.5 ml of Folin-Denis reagent and 1.0 ml of sodium carbonate solution and diluted to the mark with water. Mixed well and determined the absorbance at 760 nm after 30 min. The Plot of absorbance against mg of tannic acid taken (20 to 80µg/ml). Total tannin content was expressed as milligrams of tannic acid equivalents per gram of dried sample [27].

Results and discussions

Yield percentage

The plant samples (leaf, stem, and bark) of Manilkara hexandra were treated with seven solvents (petroleum ether, hexane, chloroform, ethyl acetate, ethanol, methanol, and double distilled water) by using a cold percolation method. These 21 crude extracts were collected and weighed. 10 g of plant samples (leaf, stem, and bark) have maximum yield present in stem, especially in alcoholic and aqueous extracts. When compared with leaf and bark of Manilkara hexandra as shown in Fig.3.

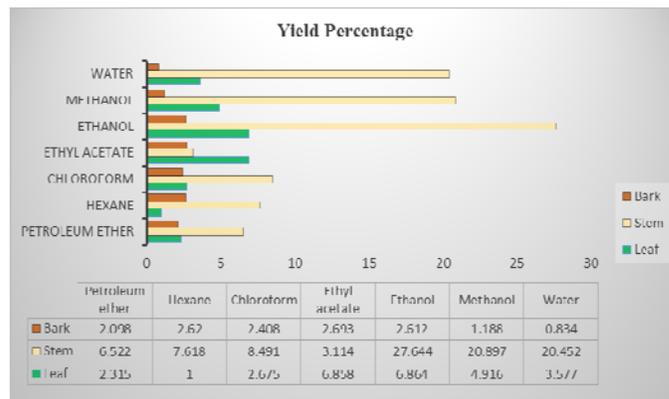


Fig 3: Yield percentage of Manilkara hexandra leaf, stem, and bark using Petroleum Ether, Hexane, Chloroform, Ethyl Acetate, Ethanol, Methanol and Water Extract.

Phytochemical Screening

Phytochemical analysis is of paramount importance in identifying new source of therapeutically and industrially valuable compound. In the present investigation of primary and secondary metabolites were qualitatively analysed using Manilkara Hexandra Leaf, Stem and Bark in various increasing polarity solvents such as petroleum ether, hexane, chloroform, ethyl acetate, ethanol, methanol and aqueous were used by the method of cold percolation. The phytochemical screening of obtained compounds (alkaloids, anthocyanin, anthroquinone, carbohydrates, coumarins, emodins, oils and fat, flavonoids, glycosides, gum, saponin, tannin, terpenoid) were reported as in Table.1.

Table 1: Phytochemical Screening of seven extracts on Manilkara Hexandra Leaf (L), Stem (S) and Bark (B).

S. No	Phyto compounds	P. E			H			C			E. A			E			M			D.D H ₂ O		
		L	S	B	L	S	B	L	S	B	L	S	B	L	S	B	L	S	B	L	S	B
1.	Alkaloid	E	-	E	G	G	P	G	E	P	-	G	P	G	E	E	E	E	G	P	P	
2.	Amino acid	-	-	-	-	-	-	-	P	-	-	-	P	G	E	G	-	E	P	-	-	-
3.	Anthocyanin	P	P	P	P	P	P	-	G	P	G	P	P	P	P	E	P	-	P	-	-	P
4.	Anthroquinone	G	G	G	G	G	G	G	P	P	P	G	E	G	G	E	P	G	P	P	P	P
5.	Carbohydrates	E	G	E	G	E	P	E	G	G	G	E	G	G	E	E	G	E	G	G	E	G
6.	Coumarins	P	P	P	G	P	P	G	G	E	P	P	P	G	P	G	P	P	P	-	-	E
7.	Emodins	G	P	P	P	G	G	P	G	G	P	P	P	P	E	G	-	G	E	-	P	-
8.	Fixed oils & Fats	P	E	E	G	G	-	E	E	P	E	G	G	E	E	P	P	P	-	-	-	-
9.	Flavonoids	E	-	-	-	-	-	-	G	G	G	E	G	E	E	G	E	E	G	E	E	P
10.	Glycosides	E	P	-	P	G	-	G	E	G	E	E	E	E	E	P	G	E	E	G	P	
11.	Gum & Musilage	E	E	G	E	G	E	-	E	E	P	E	E	E	E	G	G		E	P	E	P
12.	Phlobatannin	-	-	-	-	-	-	-	G	-	-	P	P	E	E	E	P	E	G	G	P	P
13.	Protein	G	-	-	P	P	P	G	G	E	P	P	G	E	E	E	G	E	G	P	E	G
14.	Quinone	P	-	-	-	P	-	-	P	G	P	G	G	G	E	E	P	E	E	P	P	E
15.	Resin	P	G	E	P	G	E	P	E	G	G	E	E	G	E	E	G	E	E	G	P	P
16.	Saponin	-	-	-	E	E	E	G	E	E	-	G	-	E	E	E	G	E	E	G	E	E
17.	Steroids	P	-	-	G	G	-	G	E	G	E	E	E	E	E	E	G	G	E	G	G	G
18.	Tannin	P	P	-	G	E	-	-	E	E	E	E	P	E	E	E	P	E	G	E	E	E
19.	Terpenoids	-	-	-	G	E	E	G	E	E	G	E	E	E	E	E	G	E	G	G	E	E
20.	Xanthoprotein	P	-	-	P	-	-	-	-	-	E	E	-	E	E	E	E	E	E	P	E	P

P.E= Petroleum ether, H=Hexane, C= Chloroform, E.A= Ethyl acetate, E= Ethanol, M= Methanol, DD= Double distilled water.

E = Abundant, G = Good, P = Slightly present, - = Absent

The medicinal value of plants lies in some chemical substances that have definite physiological functions in the human body. Different phytochemicals have been found to possess a wide range of medicinal properties, which may help in protection against various diseases. For example, alkaloids protect against chronic diseases; saponins protect against hypercholesterolemia and steroids and triterpenoids show the analgesic properties, anthroquinone used in dyes, flavonoids possess antioxidant properties and tannin have astringent, hemostatic, antiseptic properties.

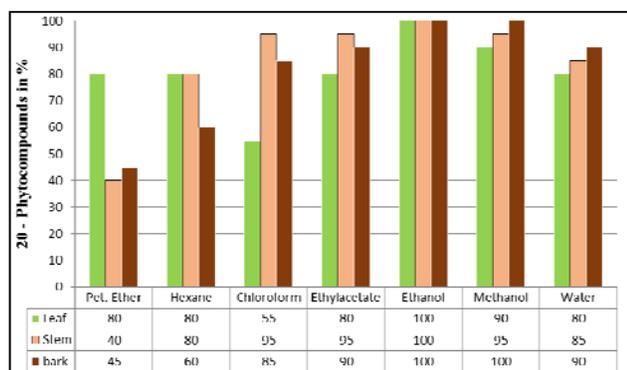


Fig 4: Phytochemicals present in screening of increasing polarity solvents.

According to the following formula, the bar chart is drawn.
 $\% \text{ of phytochemicals} = \frac{\text{Obtained number of compounds}}{\text{Total number of compounds}} \times 100$

The bar chart is discussed in fig.4 by adapting Table.1. In leaf $\geq 50\%$ of phytochemicals present from low polar to high polar solvents. Similarly, the stem and bark contains $\geq 40\%$ and 45% respectively. In general, all the low polar compounds are soluble in low polar solvents and vice & versa. The phytochemicals of the leaf are maximum in petroleum ether and hexane extracts, minimum in Chloroform, ethyl acetate, methanol and water extracts

compared with Stem and bark except for ethanol. The polarity of stem is raising from petroleum ether to ethyl acetate extract, slightly varies from methanol to water extracts compared with leaf and bark. The compound is maximum in chloroform and minimum in petroleum ether except ethanol. The phytochemicals are highly persist in bark of high polar solvents such as methanol and water correlate with leaf and stem. Out of these seven extracts, ethanolic extract of leaf, stem, and bark show maximum number (100%) of plant constituents such as alkaloids, terpenoids, saponins, flavonoids, phenol, tannins, steroids, carbohydrate, glycosides, protein, amino acids, gum & musilage and anthraquinone. Phytochemicals are naturally synthesized in all parts of the plant like bark, leaves, stem, root, flower, fruits, seeds, etc and the quantity and quality of phytochemicals present in plant parts may differ from one another [28].

Quantitative analysis

The quantitative results were evaluated between the phytochemicals such as alkaloids, flavonoids, saponin, terpenoids, phenol and tannin. The leaf, stem, and bark of Manilkara hexandra were quantified by using standard procedure of alkaloids, flavonoids, terpenoids, and saponins as shown in Table.3. The phenol and tannins were analysed by using standard chemicals (Gallic acid and Tannic acid) of calibration curve ($Y = mx + C$). The plot of graph (Fig.5 and Fig.6) represents the absorbance (nm) against concentration ($\mu\text{g/ml}$). It provides the unknown concentration of phenol and tannin for leaf, stem, and bark of Manilkara hexandra. The quantitative results were compared in Table.2.

Table 2: Comparative report for quantitative analysis.

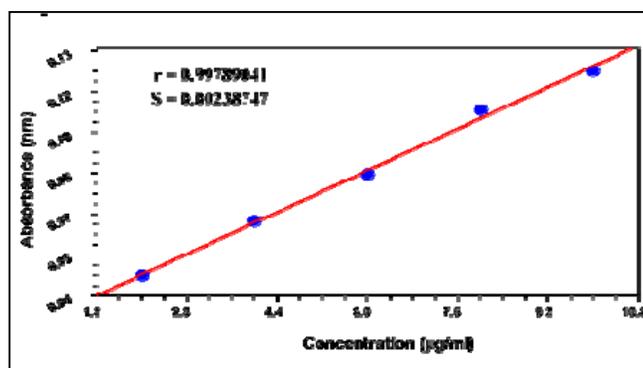
Phytochemicals of secondary metabolites	Ascending Order of plant parts
Alkaloids and Saponin	B < L < S
Terpenoids, Phenol, and Tannin	L < S < B
Flavonoids	B < S < L

L = Leaf; S = Stem; B =Bark.

Table 3: Quantitative phytochemical Analysis

S.no	Phytochemicals	Weight(mg/g)		
		Leaf	Stem	Bark
1.	Alkaloids	119.2 ± 37.8	153.8 ± 45.8	69 ± 28
2.	Flavonoids	144.2 ± 3.16	128 ± 14.66	29.7 ± 3.2
3.	Saponins	205.2 ± 50	211 ± 70.4	115.6 ± 12
4.	Terpenoids	10 ± 1.3	18.45 ± 0.75	34.0000 ± 4.1000
5.	Phenols	1.335 ± 0.0182	6.5118 ± 0.0162	9.4886 ± 0.0002
6.	Tannins	42.6273 ± 0.01868	74.1462 ± 6.0035	79.0319 ± 0.0188

Values are calculated by using Mean ± S.D in three replicates.



R= Regression value, S = Slope

Fig 5: Standard graph of phenol using gallic acid ($\mu\text{g/ml}$)

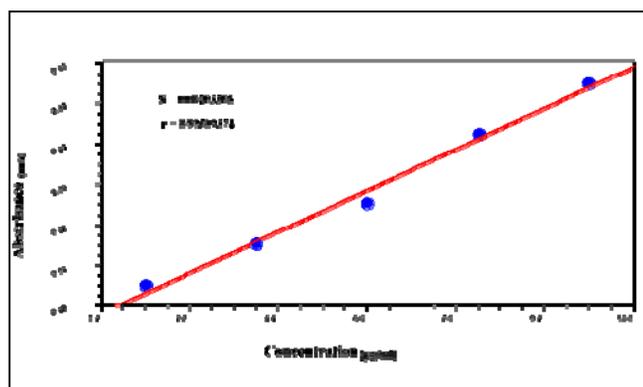


Fig 6: Standard graph of Tannin using tannic acid ($\mu\text{g/ml}$)

Conclusion:

Plants are the major source for secondary metabolites, they are meant for several biological activities in human and animals [29]. Ethanolic extract consists huge amount of secondary metabolites in leaf, stem, and bark of *Manilkara hexandra*. Alkaloids and Saponins were rich in stem, and Flavonoids in leaf. Terpenoids, phenol and tannin were abundant in bark. The stem has the highest yield compare with leaf and bark. From the above results, it concluded that the each different parts (leaf, stem, and bark) has various medicinal uses.

References

- Chessbrough M. Medicinal laboratory manual for tropical countries, Lianacre Houses. Microbiology. Butterworth-Heinemann Ltd., Linacre House, Jordan. Hill, Oxford, 2: 200-44-289-311.
- Chopra A, VV Doiphode, Ayurvedic medicine: core concept, therapeutic principles and current relevance. Medical Clinics of North America, 2002; 86:75-89.
- Joshi SG, Medicinal plants, Oxford press, Kolkata, 2000; 361-392.
- Patil KJ, Patil SV, Biodiversity of vulnerable and endangered plants from Jalgaon district of North Maharashtra. Asian J Pharma. Life Sci, 2012; 2(2):144 - 150.
- Rao R, Krishna S, Sudarshan SR. Encyclopaedia of Indian Medicine. First ed. Popular Prakashan, Bangalore, 1985; 242 - 243.
- Azadirachta indica plant species, Glo Adv Res J Environ Sci Toxicol, 2012; 1:14-17 5.
- Kumari SPK, Sridevi V, Lakshmi MVVC. Studies on Phytochemical screening of aqueous extract collected from fertilizers affected two medicinal plants. J Chem Bio Phy Sci, 2012; 2:1326-1332.
- Kharat SS, Kumkar PB, Siddhesh RR, Sonawane KS. Qualitative phytochemical screening of *Gnidia glauca* (Fresen) Gilg. Plant extract. Int J Pharm Bio Sci, 2013; 4:144-148.
- Kavitha R, Premalakshmi V. Phytochemical analysis of ethanolic extract of leaves of *Clitoria ternatea* L. Int J Pharm Bio Sci, 2013; 4:236-242.
- Dasgupta S, Parmar A, Patel H. Preliminary phytochemical studies of *Kalanchoe Gastonis-bonnieri*. Int J Pharm Bio Sci, 2013; 4:550-557.
- DE Okwu, phytochemicals and vitamins content of indigenous spices of south Eastern nigeria. J. Sustainable agriculture and environment, 2004; 6(1):30-37.
- DE Okwu, phytochemicals, vitamins and minerals contents of two Nigerian Medicinal plants, international j. Molecular medicine and advance sciences, 2005; 1:375-381.
- Manilkara hexandra information from NPGS / GRIN. Taxonomy for plants. National Germplasm Resources laboratory. Beltsville, nMaryl and : USDA, ARS, National Genetic Resources Program. Retrieved, 2009.
- Anokwuru CP, Anyasor GN, Ajibaye O, Fakoya O, Okebugwu P, Effect of Extraction Solvents on Phenolic, Flavonoid and Antioxidant activities of three Nigerian Medicinal Plants, nature and Science, 2011; 9(7).
- Evans WC. Trease and Evans, Pharmacognosy, 13 Edn. Bailliere Tindall. London, 1989; 830.
- Gokhale SB, Kokate CK, Purohit AP, A text book of Pharmacognosy, Nirali Prakshan, Pune, India, 1-50.
- Trease GE, Evans WC. A textbook of Pharmacognosy. 14th ed London: Bailliere Tindall Ltd, 1996.
- Harborne JB. A Guide to modern techniques of plant Analysis. USA: Kluwer Academic Publisher, 1998.
- Solomon Charles Ugochukwu, Arukwe Uche L, Onuoha Ifeanyi, Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G Baker, Asian journal of plant science and research, 2013; 3(3):10-13.
- Kokate CK. Practical Pharmacognosy. 4th edn. Vallabh Prakashan Publication, New Delhi, India, 1999.
- Whistler RL, BeMiller JN, Industrial Gums: Polysaccharides and their Derivatives. Academic Press, London, U.K, 1993.
- Harborne JB. Phytochemical methods, London. Chapman and Hall, Ltd, 1973; 49-188.
- Boham BA, Kocipai-Abyazan R. Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycinium* Pacific Science, 1974; 48: 458- 463.
- Dayana Jeya Leela G, Irudaya Monisha S, Anitha Immaculate A, Rosaline Vimala R, Studies on

- Phytochemical, Nutritional Analysis and Screening of *in Vitro* Biological activities of Meliadubia Leaf extract, International Journal of Scientific & Engineering Research 2016; 1.7(8).
25. Obadoni BO, PO Ochuko. Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria Global J. Pure Appl. Sci, 2001; 8: 203-208.
 26. Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem, 2003; 81:321–326.
 27. Bajaj KL, Devsharma AK. A Colorimetric Method for the Determination of Tannins in Tea. Mikrochimica Acta [Wien], 1977; 2:249-253.
 28. Tiwari P, kumar B, Kaur M, G Kaur, H Kaur *et al.* Phytochemical screening and extraction: A review. International Pharmaceutical Scientia, 2011; 1:98-106.
 29. Sofowara A, Research on medicinal plants and traditional medicine in Africa. I Aiten complement. Med, 1996; 2:365- 372.