

A Comparative Study of Physico-Chemical Parameters and Anti-Microbial Activities of Oils Extracted from Leaves of *Plectranthus Amboinicus* and *Plectranthus Rotundifolius*

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Abstract – This is a comparative study of few physico-chemical parameters and antimicrobial activities of oils extracted from two species of herbs belonging to genus *Plectranthus* viz., *Plectranthus amboinicus* and *Plectranthus rotundifolius*. In the present study, the physical parameters like melting point, density and refractive index of both the oils were compared. The chemical parameters of the oils like fatty acid content, iodine number and saponification value were also compared. The antimicrobial activities of the oils extracted were compared by performing agar well-diffusion method against two Gram-positive pathogenic bacteria namely, *Staphylococcus aureus* and *Bacillus subtilis* and two Gram-negative pathogenic bacteria namely, *Escherichia coli* and *Pseudomonas aeruginosa*.

Keywords – Physico-Chemical Parameters, *Plectranthus*, Agar Well-Diffusion Method, Gram-Positive, Gram-Negative.

I. INTRODUCTION

India is enriched with a varied heritage of medicinal plants owing to its favourable geographical conditions. This fact is being exploited traditionally since Vedic ages in India. The Indian Ayurvedic system of treatment is recently being accepted world-wide. There are about 45,000 plant species in India, with concentrated hotspots in the regions of Eastern Himalayas, Western Ghats and Andaman and Nicobar islands. The Western Ghats of India are rich with a variety of plants of medicinal significance. Many herbs can be used domestically in treatment of various diseases like cough, cold, stomach-related disorders and so on. Two such plants namely, *Plectranthus amboinicus* and *Plectranthus rotundifolius*, adequately available in the Western Ghats are the subjects of the present study. *Plectranthus amboinicus*, identified as *Coleus amboinicus* initially, is a semi-succulent perennial plant in the family Lamiaceae with a pungent oregano-like flavor and odor. *Plectranthus amboinicus* Lour. (Lamiaceae) known as Country Borage, Indian borage in English and Patta Ajwain, Patharcure in Hindi. Leaves roots and stems are used as carminative, digestive, stomachic, anthelmintic, expectorant, diuretic, otalgia, anorexia, diarrhea and cholera especially in children, convulsions epilepsy, chronic asthma, bronchitis, renal and vesical calculi and malarial fever (B. Sreedharren *et al.*, 2010). *Plectranthus rotundifolius* is a perennial herbaceous plant of the mint family (Lamiaceae) native to tropical Africa. It is cultivated for its edible tubers primarily in West Africa, as well as more recently in parts of Asia, especially India, Sri Lanka, Malaysia and Indonesia. *P.*

rotundifolius is a rich source of carbohydrates, protein, fat and fiber content and has nutritional and anti-nutritional components which show medicinal property and are utilized for health care and pharmaceutical application and *P. rotundifolius* tuber contains nutritional and large amount of secondary metabolites which helps in improving body immune system and defense against diseases (M. Hema Priya and S. Anbuselvi, 2013). *P. rotundifolius* shows presence of different functional groups such as alcohols, phenols, amines, alkanes, aldehydes, carboxylic acid, iso cyanides, alkyne, isocyanate, ketones, aromatics, phenols, tertiary and primary alcohols and chloro compounds (S. Manikandan *et al.*, 2016). Physical parameters like apparent density, refractive index, solid fat content, melting point, boiling point, etc. and chemical parameters like peroxide value, iodine value, saponification value, etc. of oils are studied for understanding the nature of oils extracted from various plants. Anti-microbial activity can be defined as any biological activity that acts against bacteria, fungi, or virus. There are many plant-based extracts available that have been proven to have antimicrobial properties. Praveena Bhatt *et al.*, (2012) studied antioxidant and antibacterial activities in the different solvent extracts of the leaves of Indian Borage (*Plectranthus amboinicus*) and reported that extraction of phenolic compounds is highest when methanol or aqueous methanol is used and *P. amboinicus* showed higher antioxidant and antibacterial activity Gram positive and Gram-negative bacteria and fungi.

II. MATERIALS AND METHODS

A. Soxhlet Extraction of Oils from Leaves of *P. Amboinicus* and *P. Rotundifolius*:

a. Sample Preparation:

Fresh leaves of *P. amboinicus* and *P. rotundifolius* were acquired from the near localities of Puthenvelikara and were botanically identified and verified in the Life Sciences Department of Presentation College of Applied Sciences, Puthenvelikara. The respective leaves were washed with tap water 2-3 times and carefully blotted dry by spreading on blotting paper. 500 g each of the leaves were weighed and subjected to 94% drying by placing them in hot air oven at 50°C for 8hrs. The total dry weight was found to be 30 g.

b. Soxhlet Extraction:

In the present study, 80% methanol was used as extraction solvent and the extraction of oils from both *P. amboinicus* and *P. rotundifolius* was done at 60°C

temperature for 24 hrs (Roshan D. Patel *et al.*, 2010). Being immiscible and lighter than methanol, the volatile oils separated out as upper layer. The oils were then separated from methanol by evaporation of methanol at 40°C and collected in small bottles, sealed, labelled and stored in light-resistant vials at 4-6°C for further use. The oil weights were recorded and percentage yield of extracted oils was calculated.

B. Calculating the percentage yield of extracted oils:

The percentage yield of extracted oils from both *P. amboinicus* and *P. rotundifolius* were calculated using the following formula:

$$\text{Percentage yield of oil} = (\text{weight of oil without solvent} / \text{total dry weight of leaf sample})$$

C. Comparing the Physical Parameters of Extracted Oils:

The physical parameters – density, refractive index and melting point of the extracted oils were compared in the present study.

a. Density (ρ):

In the present study, the densities of both the oils was calculated using the following method (Jack Brubaker, 2003). The density of oil was calculated by dividing the mass of oil in grams to volume in millilitres using the following formula:

$$\text{Density } (\rho) = M / V \dots \text{where } M = \text{mass of oil in grams; } V = \text{volume of oil in cm}^3$$

b. Refractive Index (n):

In the present study, the oils extracted from both *P. amboinicus* and *P. rotundifolius* were subjected to Abbe Refractometer (WYA) to note down their respective refractive indices.

c. Melting Point (T_m):

In the present study, melting points of the oils extracted from both *P. amboinicus* and *P. rotundifolius* were calculated by the procedure by British Nutrition Foundation 2004 (Energy and Nutrients 1995).

D. Comparing the Chemical Parameters of Extracted Oils:

The chemical parameters – fatty acid content, iodine value and saponification value of the extracted oils were compared in the present study.

a. Fatty Acid Content:

A small quantity of free fatty acid is usually present in oils along the triglycerides. The free fatty acid number / acid value. It increases during storage. The keeping quality of oil therefore relies upon the free fatty acid content. The fatty acid content was calculated using the following formula:

$$\text{Acid value (mg KOH / g)} = (\text{Titre value} \times \text{Normality of KOH} \times 56.1) / \text{weight of the sample}$$

b. Iodine value:

The iodine numbers were calculated using the following the formula:

$$\text{Iodine number} = \{(B - S) \times N \times 12.69\} / \text{g sample}$$

c. Saponification Value:

The saponification values were calculated using the following formula:

$$\text{Saponification value} = 28.05 \times (\text{titre value of blank} - \text{titre value of sample})$$

E. Comparing the Anti-Microbial Properties of the Oils by Agar Well-Diffusion Method:

The agar well-diffusion bioassay (Rasoanaivo & Ratsimamanga M. Urverg, 1993) was used to determine the growth inhibition of bacteria by both the extracted plant oils.

The following Gram-positive bacteria - *Staphylococcus aureus* and *Bacillus subtilis* and the following Gram-negative bacteria - *Escherichia coli* and *Pseudomonas aeruginosa* were used as test organisms. These bacteria were maintained at 4°C on Nutrient agar (NA) medium plates. Agar plates were prepared by pouring 20 ml NA medium into sterile petri dishes and allowed to set. Nutrient broth cultures of all the test organisms were maintained at overnight incubation at 48°C. After overnight incubation, 1ml of each of the test cultures was seeded onto the base plates and subjected to incubation. Different volumes of both the extracted oils ranging from 50 µl- 500 µl was inoculated in wells formed on the inoculated base agar plates. Each of the oil was tested with all the four test organisms and the duplicates of each plate were maintained. The Neem oil of respective volumes was used as biological control and ampicillin of respective volumes was used as antibiotic control. The plates were evaluated after incubation at 37°C for 18 hrs. By measuring diameter of the zone of inhibition (mm) of bacterial growth around the wells in each of the bacterial plates. Duplicates were maintained for each plate. Anti-bacterial activities of both oils were compared by calculating the net area of zone of inhibition in each bacterial plate. Net area of zone of inhibition was calculated in mm² using the formula:

$$\text{Net area of zone of inhibition (mm}^2\text{)} = \pi(A/2)^2 - \pi(B/2)^2$$

The observations were tabulated as follows:

I. Anti-microbial effects of oil extracted from *P. amboinicus*:

Plate No.	Bacterium inoculated on agar plate	Volume of samples inoculated in wells (µl)	Diameter of zone of inhibition (mm) in wells containing		
			1-Test oil	2-Neem oil	3-Ampicillin
1.	<i>S. aureus</i>	50	1.10	1.00	2.0
2.	<i>S. aureus</i>	100	1.80	1.00	2.2
3.	<i>S. aureus</i>	150	1.50	1.00	2.3
4.	<i>S. aureus</i>	200	1.60	1.00	2.4
5.	<i>S. aureus</i>	250	1.60	1.00	-
6.	<i>S. aureus</i>	300	1.70	1.00	-
7.	<i>S. aureus</i>	350	2.00	1.00	3.00
8.	<i>S. aureus</i>	400	2.50	1.00	3.10
9.	<i>S. aureus</i>	450	1.50	1.00	1.10
10.	<i>S. aureus</i>	500	1.20	1.00	1.20
11.	<i>B. subtilis</i>	50	-	1.50	1.3
12.	<i>B. subtilis</i>	100	-	1.50	1.3
13.	<i>B. subtilis</i>	150	-	1.50	1.4
14.	<i>B. subtilis</i>	200	-	1.50	-
15.	<i>B. subtilis</i>	250	1.6	1.50	1.4
16.	<i>B. subtilis</i>	300	2.00	1.00	2.20
17.	<i>B. subtilis</i>	350	2.70	1.00	2.30
18.	<i>B. subtilis</i>	400	2.50	-	1.50
19.	<i>B. subtilis</i>	450	1.90	-	2.00
20.	<i>B. subtilis</i>	500	1.70	-	2.00
21.	<i>E. coli</i>	50	-	-	2.50
22.	<i>E. coli</i>	100	-	-	1.80
23.	<i>E. coli</i>	150	-	-	2.50
24.	<i>E. coli</i>	200	-	-	1.00
25.	<i>E. coli</i>	250	0.90	1.20	1.90
26.	<i>E. coli</i>	300	-	-	-
27.	<i>E. coli</i>	350	1.90	-	2.90
28.	<i>E. coli</i>	400	2.30	2.00	3.00
29.	<i>E. coli</i>	450	2.10	2.00	3.00
30.	<i>E. coli</i>	500	2.00	2.00	3.10
31.	<i>P. aeruginosa</i>	50	1.20	1.00	1.50
32.	<i>P. aeruginosa</i>	100	1.30	1.00	1.60
33.	<i>P. aeruginosa</i>	150	-	1.00	1.60
34.	<i>P. aeruginosa</i>	200	1.50	1.00	-
35.	<i>P. aeruginosa</i>	250	1.50	1.00	1.50
36.	<i>P. aeruginosa</i>	300	-	1.20	1.30
37.	<i>P. aeruginosa</i>	350	1.10	1.00	1.30
38.	<i>P. aeruginosa</i>	400	1.10	-	1.30
39.	<i>P. aeruginosa</i>	450	1.20	-	1.30
40.	<i>P. aeruginosa</i>	500	1.20	-	1.30

II. Anti-microbial effects of oil extracted from *P. rotundifolius*:

Plate No.	Bacterium inoculated on agar plate	Volume of samples inoculated in wells (µl)	Diameter of zone of inhibition (mm) in wells containing		
			1-Test oil	2-Neem oil	3-Ampicillin
1.	<i>S. aureus</i>	50	-	-	2.50
2.	<i>S. aureus</i>	100	-	-	1.80
3.	<i>S. aureus</i>	150	-	-	2.50
4.	<i>S. aureus</i>	200	-	-	1.00
5.	<i>S. aureus</i>	250	0.90	1.20	1.90
6.	<i>S. aureus</i>	300	-	-	-
7.	<i>S. aureus</i>	350	1.90	-	2.90
8.	<i>S. aureus</i>	400	2.30	2.00	3.00
9.	<i>S. aureus</i>	450	2.10	2.00	3.00
10.	<i>S. aureus</i>	500	2.00	2.00	3.10
11.	<i>B. subtilis</i>	50	1.20	1.00	1.50
12.	<i>B. subtilis</i>	100	1.20	1.00	1.50
13.	<i>B. subtilis</i>	150	1.30	1.00	1.60
14.	<i>B. subtilis</i>	200	-	1.00	1.60
15.	<i>B. subtilis</i>	250	1.50	1.00	-
16.	<i>B. subtilis</i>	300	1.50	1.00	1.50
17.	<i>B. subtilis</i>	350	-	1.20	1.30
18.	<i>B. subtilis</i>	400	1.10	1.00	1.30
19.	<i>B. subtilis</i>	450	1.10	-	1.30
20.	<i>B. subtilis</i>	500	1.20	-	1.30
21.	<i>E. coli</i>	50	1.20	-	1.30
22.	<i>E. coli</i>	100	-	1.50	1.3
23.	<i>E. coli</i>	150	-	1.50	1.4

Plate No.	Bacterium inoculated on agar plate	Volume of samples inoculated in wells (µl)	Diameter of zone of inhibition (mm) in wells containing		
			1-Test oil	2-Neem oil	3-Ampicillin
24.	<i>E. coli</i>	200	-	1.50	-
25.	<i>E. coli</i>	250	1.6	1.50	1.4
26.	<i>E. coli</i>	300	2.00	1.00	2.20
27.	<i>E. coli</i>	350	2.70	1.00	2.30
28.	<i>E. coli</i>	400	2.50	-	1.50
29.	<i>E. coli</i>	450	1.90	-	2.00
30.	<i>E. coli</i>	500	1.70	-	2.00
31.	<i>P. aeruginosa</i>	50	1.10	1.00	2.0
32.	<i>P. aeruginosa</i>	100	1.80	1.00	2.2
33.	<i>P. aeruginosa</i>	150	1.50	1.00	2.3
34.	<i>P. aeruginosa</i>	200	1.60	1.00	2.4
35.	<i>P. aeruginosa</i>	250	1.60	1.00	-
36.	<i>P. aeruginosa</i>	300	1.70	1.00	-
37.	<i>P. aeruginosa</i>	350	2.00	1.00	3.00
38.	<i>P. aeruginosa</i>	400	2.50	1.00	3.10
39.	<i>P. aeruginosa</i>	450	1.50	1.00	1.10
40.	<i>P. aeruginosa</i>	500	1.20	1.00	1.20

III. RESULTS

A. Calculating the Percentage Yield of Extracted Oils:

After calculating the percentage yields of oils after soxhlet extraction from leaves of *P. amboinicus* and *P. rotundifolius*, it was found that the percentage yield of oil extracted from *P. amboinicus* was found to be 53.26 % and the percentage yield of oil extracted from *P. rotundifolius* was found to be 55.35 %.

B. Comparing the Physical Parameters - Density, Refractive Index and Melting Point of the Oils:

After comparing the physical parameters - density, refractive index and melting point of the oils after soxhlet extraction from leaves of *P. amboinicus* and *P. rotundifolius*, the following results were obtained:

a. Density (ρ):

The density of oil extracted from leaves of *P. amboinicus* was found to be 0.9207 ± 0.0001 g/cm³. The density of oil extracted from leaves of *P. rotundifolius* was found to be 1.250 ± 0.0001 g/cm³.

b. Refractive Index (n):

The refractive index of oil extracted from leaves of *P. amboinicus* was found to be 1.34 units. The refractive index of oil extracted from leaves of *P. rotundifolius* was found to be 1.37 units.

c. Melting Point (T_m):

The melting point of oil extracted from leaves of *P. amboinicus* was found to be $125 \pm 2^\circ\text{C}$. The melting point of oil extracted from leaves of *P. rotundifolius* was found to be $130 \pm 2^\circ\text{C}$.

C. Comparing the Chemical Parameters – Fatty Acid Content, Iodine Value and Saponification Value of the Oils:

After comparing the chemical parameters – fatty acid content, iodine value and saponification value of the oils after soxhlet extraction from leaves of *P. amboinicus* and *P. rotundifolius*, the following results were obtained:

i. Fatty Acid Content:

The fatty acid content of oil extracted from leaves of *P. amboinicus* was found to be 108.61 mg KOH/g. The fatty

acid content of oil extracted from leaves of *P. rotundifolius* was found to be 117.83 mg KOH/g.

ii. Iodine Value:

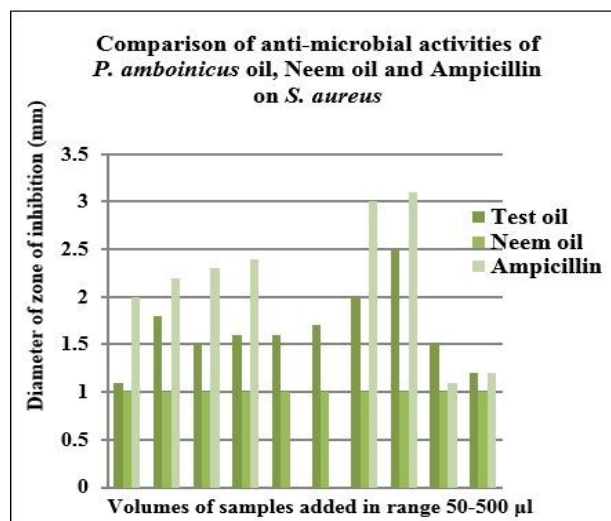
The iodine value of oil extracted from leaves of *P. amboinicus* was found to be 176.0/g. The iodine value of oil extracted from leaves of *P. rotundifolius* was found to be 185.0/g.

iii. Saponification Value:

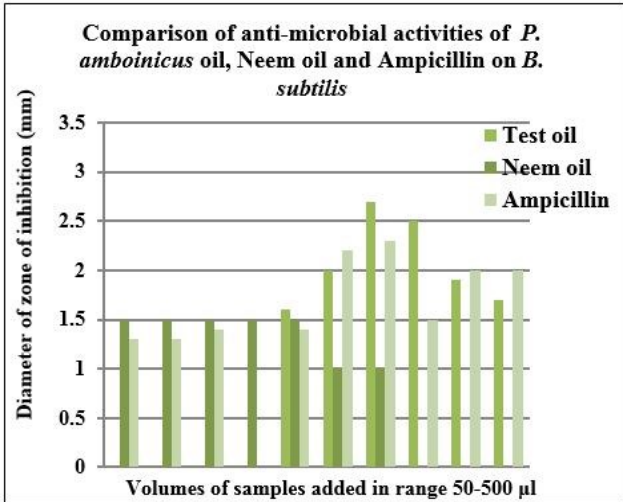
The saponification value of oil extracted from leaves of *P. amboinicus* was found to be 126.0 units. The saponification value of oil extracted from leaves of *P. rotundifolius* was found to be 133.0 units.

D. Comparing the Anti-Microbial Properties of the Oils by agar Well-diffusion Method:

The results of anti-microbial effects of the oils extracted from leaves of *P. amboinicus* and *P. rotundifolius* in comparison to anti-microbial effects of Neem oil and ampicillin were studied. The oil from *P. amboinicus* was seen to show more anti-microbial activity against Gram positive bacteria- *S. aureus* and *B. subtilis* than against the Gram negative ones. The results were graphically represented as follows:



Graph 1. Comparison of anti-microbial activities of *P. amboinicus* oil, Neem oil and Ampicillin on *S. aureus*



Graph 2. Comparison of anti-microbial activities of *P. amboinicus* oil, Neem oil and Ampicillin on *B. subtilis*

P. amboinicus oil was seen to have highest anti-microbial activity against *B. subtilis*

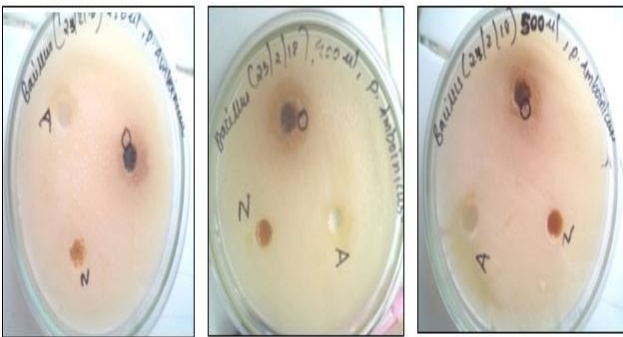
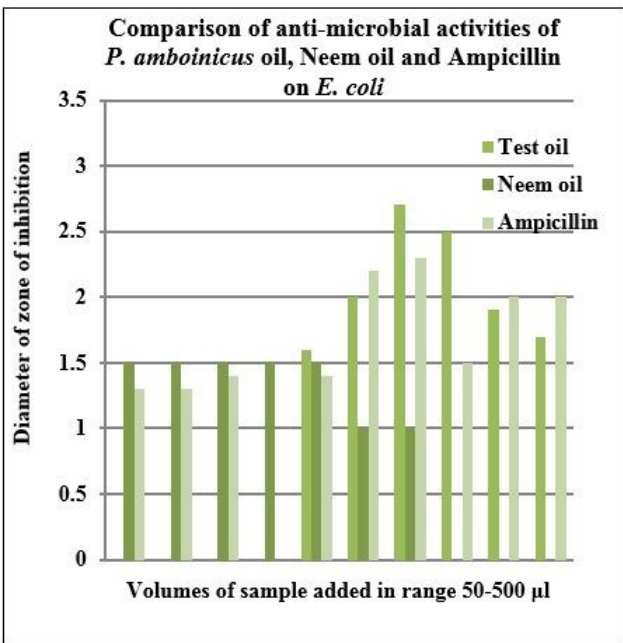
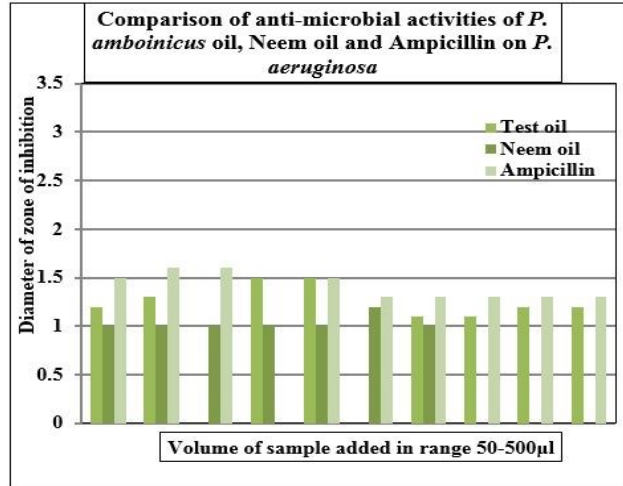


Fig. 1. Antimicrobial activity of *P. amboinicus* oil against *B. subtilis*

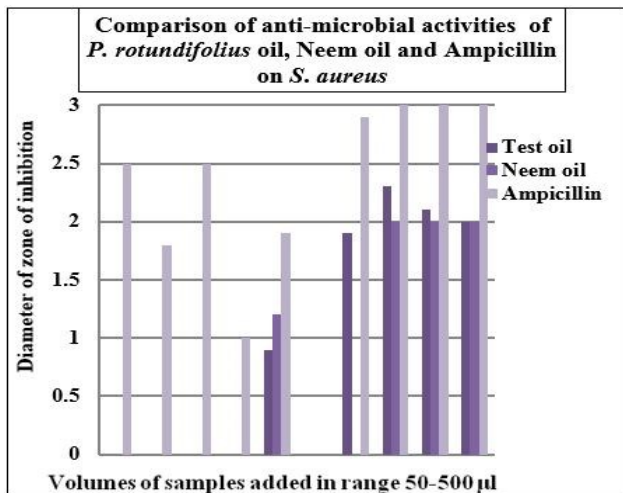


Graph 3. Comparison of anti-microbial activities of *P. amboinicus* oil, Neem oil and Ampicillin on *E. coli*

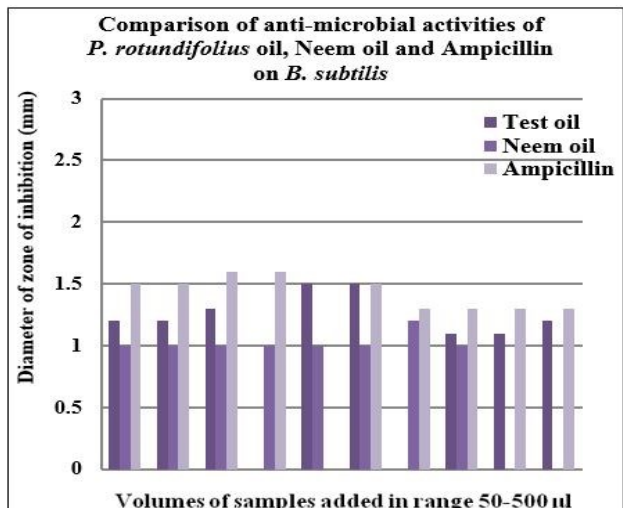


Graph 4. Comparison of anti-microbial activities *P. amboinicus* oil, Neem oil and Ampicillin on *P. aeruginosa*

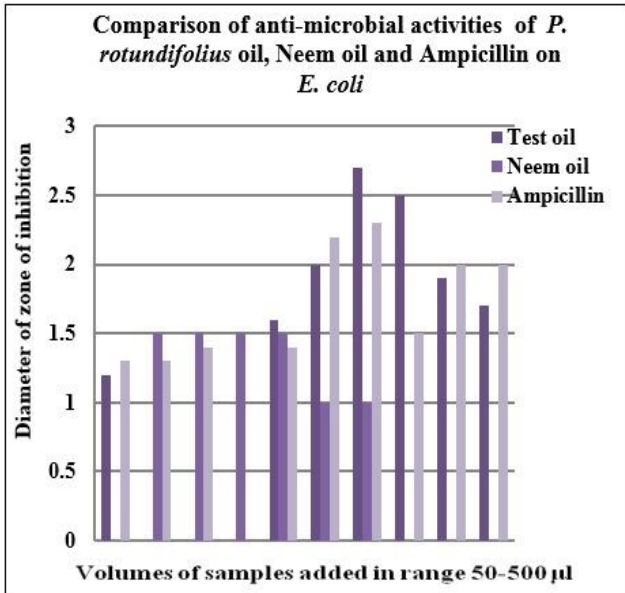
The oil from *P. rotundifolius* was seen to show more anti-microbial activity against Gram negative bacteria - *E. coli* and *P. aeruginosa* than against the Gram positive ones. The results were graphically represented as follows:



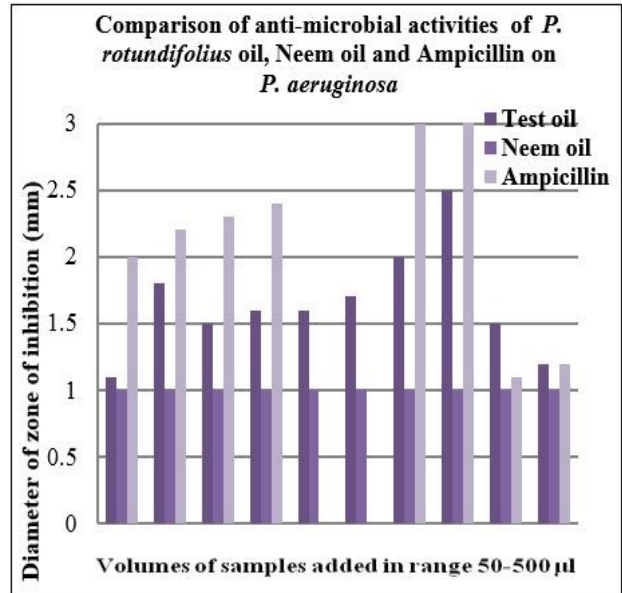
Graph 5. Comparison of anti-microbial activities of *P. rotundifolius* oil, Neem oil and Ampicillin on *S. aureus*



Graph 6. Comparison of anti-microbial activities of *P. rotundifolius* oil, Neem oil and Ampicillin on *B. subtilis*



Graph 7. Comparison of anti-microbial activities of *P. rotundifolius* oil, Neem oil and Ampicillin on *E. coli*



Graph 8. Comparison of anti-microbial activities of *P. rotundifolius* oil, Neem oil and Ampicillin on *P. aeruginosa*

P. rotundifolius oil was seen to have highest anti-microbial activity against *E. coli*.

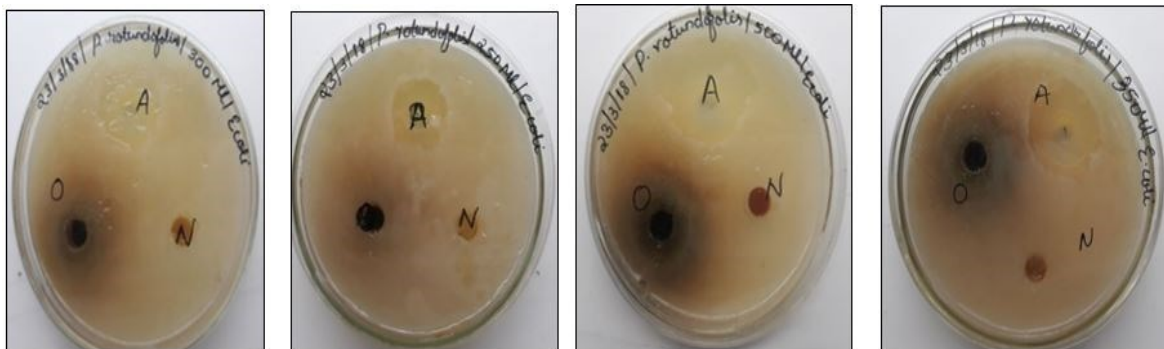


Fig. 2: Antimicrobial activity of *P. rotundifolius* oil against *E. coli*.

IV. DISCUSSION & CONCLUSION

It can be stated from the present study that, the physico-chemical parameters of *P. rotundifolius* oil were somewhat similar to those of *P. amboinicus*. There were only slight variations in values observed. Like the leaves of *P. amboinicus*, the leaves of *P. rotundifolius* can also be exploited for various purposes like therapeutic purposes, in aroma therapy, etc. The leaves of *P. rotundifolius* were also seen to show a moderately high activity against Gram-negative bacteria, especially *E. coli*, in contrast to *P. amboinicus* which showed low activity against Gram-negative bacteria. It was also seen to show a much better anti-microbial activity than the widely accepted anti-microbial substance-Neem oil. Thus, the use of leaves of *P. rotundifolius* can be made in use to treat diseases like neonatal meningitis, urinary tract infections, hemorrhagic colitis, Crohn's disease, etc. The oil from the leaves of *P. rotundifolius* can be used in combination with the

prevailing medicines available in global markets to present a better synergistic effect against these diseases. Hence, it is recommended that the people all around the globe make use of not only the tubers of *P. rotundifolius* but also its leaves. This can in turn increase the demand for *P. rotundifolius* in global markets. The farmers in Africa and south-east Asia may cultivate *P. rotundifolius* for obtaining both tubers and leaves. This can help the nations' agricultural sectors to grow. The cultivation, use and sale of leaves of *P. rotundifolius* may also help overcome the losses that may happen to the farmers at times of low productivity of tubers and during off-seasons when the yield of the tubers is low. Tissue-cultured *P. rotundifolius* can also be propagated to make them commercially available when more in demand, boosting up the tissue-culture sectors. The researchers may develop various value-added preparations from its leaves, ultimately improving a country's scientific, technological, industrial and economic sectors, leading to an overall development of the country.

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I dedicate this work to my parents and other family members for their constant support.

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