A Comparative Evaluation of Cold Maceration, Soxhlation and Ultra Sound Assisted Extraction for Phytochemical Screening of Whole Plnt of Blumea Lacera(Burm.f.) Dc. Along With It's Antibacterial Effect With Respect to Catharanthus Roseus (L.) G. Don.

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Abstract

Background

Here the experiment was performed with two medicinally important traditional plants extracts like *Blumea lacera* (Burm.f.) DC. And *Catharanthus roseus* (L.) G. Don.. Three distinct extraction procedure like cold maceration, ultra sound sonication and soxhlation were used for extraction of *Blumea lacera* (Burm.f.) DC. and compared the variation of phytoconstituents extracted through those traditional and modern methods of extraction. Also there were a systematic examination of *Blumea lacera* (Burm.f.) DC. extract effectivity against a diverse range of pathogenic bacteria, including Gram-positive and Gram-negative, with respect to *Catharanthus roseus* (L.) G. Don.extract.These analysis highlighting the potency of traditional medicinal plants as sources of novel antibacterial agents.

Results

Maximum greenish brown extract was obtained by Soxhlation of *Blumea lacera* (Burm.f.) DC. whole plant that was 8.8% but maximum phytoconstituents were extracted by ultrasound assisted sonication. It was observed that antibacterial activity of 100 mg/litre of whole plant Soxhlate extract of *Blumea lacera* (Burm.f.) DC.was more than *Catharanthus roseus* (L.) G. Don. against *Escherichia Coli* (ATCC-8739). Again whole plant extract of *Blumea lacera* (Burm.f.) DC. 100mg/litre was effective against *Staphylococcus aureus* (ATCC-6538) and *Salmonella abony* (NCTC-6017) and 50 mg/litre was also effective against *Staphylococcus aureus* (ATCC-6538) where *Catharanthus roseus* (L.) G. Don. extract was ineffective.

Conclusion

This study validates the most efficient extraction technique of *Blumea lacera* (Burm.f.) DC. to get specific phytoconstituents and notable antibacterial efficacy with specific concentrations against specific pathogenic bacteria with respect to *Catharanthus roseus* (L.) G. Don. This study showed that the quality and quantity of yield of each extraction technique derived extract may varied. Here the highest yield was obtained by soxhlation and lowest yield was obtained by cold maceration technique. With respect to *Catharanthus roseus* (L.) G. Don., *Blumea lacera* (Burm.f.) DC.was more effective against both Gram-positive and Gram-negative bacteria, due to it's large number of phytoconstituents like alkaloids, glycosides, steroids, terpenoids etcetera. These findings highlight the need to choose specific plant extracts with specific concentrations to target particular pathogens effectively.

BACKGROUND

Blumea lacera (Burm.f.) DC. is the plant belonging to the asteraceae family, found in tropical and subtropical regions of Asia, particularly in Southeast Asia and the Indian subcontinent. They are charecterized by their aromatic leaves and small, daisy like flowers. These plants often thrive in disturbed habitats such as roadsides and clearings [1]. The plant is a tiny tree with a strong camphorous smell that can reach heights of 3–4 meters in lowlands, riverbeds, valleys, mountainous areas, fields, beneath forests, and roadsides [2]. This plant is used in traditional medicine and have applications in herbal remedies, including their ecological roles such as providing habitat for various insects [3]. The anticancer effects have been investigated, though the Indian species of Blumea have not been disclosed [4]. In traditional medicine, the plant, which has a potent turpentine scent, is employed as a diuretic and antihelminthic. Numerous biological properties of the plant, including astringent, stimulant, anthelmintic, antibacterial, anti-inflammatory, and diuretic, are well-known [5]. In the other hand*Catharanthus roseus* (L.) G. Don. (family Apocynaceae) is the well-known periwinkle plants, which are primarily native to the temperate regions of Europe, northwest Africa, and southwest and central Asia. *Catharanthus roseus* (L.) G. Don. has been valued in traditional medicine worldwide—used in India for wasp stings, in Hawaii to stop bleeding, and in China as an astringent, diuretic, and cough remedy. This plant

is also called *Vinca rosea*, *Ammocallis rosea*,or *Lochnera rosea.* It's known English names are Cape Periwinkle, Rose Periwinkle, Rosy Periwinkle, and Old Maid. Extensively cultivated in northern India to meet demand in traditional medicine and the pharmaceutical industry, its growth is often hindered by soil salinity. The plant is a latex-containing, erect or spreading herb or small shrub [6]. Vinca alkaloids have a long history of use in managing conditions like diabetes and hypertension, with some of their derivatives also employed as disinfectants. Their most notable impact, however, is in oncology, where they serve as powerful chemotherapeutic agents. *Catharanthus roseus* (L.) G. Don. has a long-standing role in traditional medicine, especially in Europe, where it was used for centuries as a folk remedy for diabetes. It's enduring use highlights it's importance and value in natural and herbal healing practices [7]. These diverse uses reflect its widespread therapeutic significance across cultures [8].

RESULT

Organoleptic Evaluation

Table 1:Organoleptic properties of dried powderof whole plant

Serial number	Parameters	Result	
		Blumealacera (Burm.f.) DC	*Catharanthus roseus* (L.) G. Don.
1	Colour	Greenish Brown	Green
2	Odour	Characteristic	Characteristic
3	Taste	Bitter	Bitter

Qualitative Evaluation

Table 2: Colour, Nature and % Yield of Whole Plant Extract of *Blumealacera* (Burm.f.) DC

SL NO	COLOUR	NATURE	PERCENTAGE OF YIELD (% Weight/Weight)		
			COLD MACERATION	SOXHLATION	ULTRA SOUND SONICATION
1.	DARK GREEN	SMOOTH	5.6	8.8	7

Table 3: Result of Physicochemical Evaluation of Whole Plant Extract of *Blumea lacera* (Burm.f.) DC [Fig 5]

SL NO	PHYTOCONSTITUTES	TEST PROCEDURE	OBSERVATION		
			COLD MACERATION EXTRACT	ULTRASOUND ASSISTED EXTRACT	SOXHLATION EXTRACT
1.	ALKALOIDS	**Dragendorff's test**	+	+	+
		Mayer's test	–	–	–
		Wagner's test	+	+	+
		Hager's test	+	+	+
2.	CARBOHYDRATES	**Molisch's test**	–	+	+
		Barfoed's reagent	–	+	–
3.	TANNINS	**Ferric chloride test**	–	–	–
4.	STARCH	**Iodine test**	–	–	–
5.	STEROIDS AND TRITERPENOIDS	**Liebermann-Burchard test**	–	+	+
		Salkowski test	–	+	–
6.	CARDIAC GLYCOSIDES	**Baljet's test**	+	+	+
		Legal's test	–	+	+
7.	PROTEINS	**Xanthoproteic test**	–	+	–
		Biuret test	–	–	–

(+) present, (-) absent

Antibacterial Effect of *Blumea lacera* (Burm.f.) DC with respect to *Catharanthus roseus* (L.) G. Don

In this experiment the cork borer method was used to determine the *in-vitro* comparative antibacterial activity for whole plant extract of *Blumea lacera* (Burm.f.) DC. with respect to *Catharanthus roseus* (L.) G. Don.. It was observed that *Escherichia Coli* (ATCC-8739) was sensitive against 100 mg/litre of whole plant extract of *Blumea lacera* (Burm.f.) DC and *Catharanthus roseus* (L.) G. Don. [Fig 6]. It was also observed that *Staphylococcus aureus* (ATCC-6538) and *Salmonella abony* (NCTC-6017) were sensitive against 100 mg/litre of whole plant extract of *Blumealacera* (Burm.f.) DC though *Catharanthus roseus* (L.) G. Don. was inefficient to prevent their growth.

Table 4: Sensitivity Test of Bacterial Strain against Plant extract

SL NO	Bacteria used	Dose applied	Inhibition zone diameter for Oxytetracycline Hydrochloride (millimetre)	Inhibition zone diameter for *Blumealacera* (Burm.f.) DC (millimetre)	Inhibition zone diameter for *Catharanthus roseus* (L.) G. Don. (millimetre)
1.	***Escherichia coli*** (ATCC-8739)	**H**	15	13	11
		L	6	0	0
2.	***Staphylococcus aureus*** (ATCC-6538)	**H**	20	9	0
		L	12	3	0
3.	***Pseudomonas aeruginosa*** (ATCC-9027)	**H**	15	0	0
		L	5	0	0
4.	***Salmonella abony*** (NCTC-6017)	**H**	13	11	0
		L	7	0	0

H=100 milligram /litre, **L**=50 milligram /litre

Table 5: *In-vitro* comparative antibacterial activity against a standard antibiotic Oxytetracycline Hydrochloride for whole plant extract of *Blumea lacera* (Burm.f.) DC. with respect to *Catharanthus roseus* (L.) G. Don. was evaluated using Anova: Single Factor where was no significant difference ($p<0.05$)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	93	11.625	27.41071429
Column 2	8	36	4.5	31.14285714
Column 3	8	11	1.375	15.125

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	441.5833333	2	220.7916667	8.990063015	0.001511640732	3.466800112
Within Groups	515.75	21	24.55952381			
Total	957.3333333	23				

DISCUSSION

Nature is a rich source of medicinal plants with huge variety. In the field of pharmacy, plant derived antibacterial agents are very potential. Plant derived antibacterial agents are generally different types of secondary metabolites which may disrupt bacterial cell walls, inhibit enzymes, or interfere with microbial metabolism [9].

Many previous studies concluded that extraction of plants with organic solvent is most efficient than other solvents to extract out maximum secondary metabolites which are mainly acts as antibacterial components of plants. Specially to extract aromatic and saturated organic constituents, ethanol, methanol or other organic solvents are essential [10]. Due to this reason in this experiment methanol was choosed as solvent in all kind of traditional and modern method of extractions.

Extraction of phytoconstituents from medicinal plant is very demanding part in the field of pharmacognosy. To extract different metabolites from different parts of plant or whole plant, varieties of extraction techniques are used. The results of those extractions are very important in the field of pharmaceutical research work. Always it's an initial step of photochemistry to choose the right method of extraction for desired outcomes. Competent extraction technique should that are simple, economic, environment friendly, faster process to achieve maximum yield within short time without deviation any therapeutic effects [11]. Here in case of *Blumea lacera* (Burm.f.) DC. maximum extract was obtained by Soxhlation of whole plant with restoration of its antibacterial effectivity [Table 4] but maximum phytoconstituents were extracted by ultrasound assisted sonication within very short time [Table 3].

Antibacterial agents which remain present into the plants can be lost by continuous heating process during extraction [12]. But in this experiment Soxhlet extract was selected to study the antibacterial effectivity of extract. The result of that experiment proved that the antibacterial effectivity may not completely abolish in spite of continuous heating.

Here the experiment significantly provide an information about *in-vitro* antibacterial effectivity of *Blumea lacera* (Burm.f.) DC., an medicinally important traditional plant with respect to another medicinally important plant *Catharanthus roseus* (L.) G. Don. In this experiment *Blumea lacera* (Burm.f.) DC. showed inhibited zone against both gram positive and gram negative bacteria but in the other hand *Catharanthus roseus* (L.) G. Don. showed effectivity only against gram negative bacteria [Fig. 6]. Even the zone of inhibition of *Blumea lacera* (Burm.f.) DC. was more than *Catharanthus roseus* (L.) G. Don. [Table 4].

CONCLUSION

In this experiment the results of the three distinct extraction techniques included traditional and modern method of extraction with whole plant of *Blumea lacera* (Burm.f.) DC., were compared and subjected to preliminary phytochemical screening, which showed that the colour and percentage of yield along with extracted phytoconstituents were varied. Investigating the synergistic potential with broad spectrum antibiotics may also offer a new avenue to utilize it's antibacterial efficacy. The assessment of the antibacterial activities of *Blumea lacera* (Burm.f.) DC. with respect to *Catharanthus roseus* (L.) G. Don. against various pathogenic bacteria revealed notable efficiency of *Blumea lacera* (Burm.f.) DC.than *Catharanthus roseus* (L.) G. Don. with particular concentration. *Blumea lacera* (Burm.f.) DC. demonstrated particularly robust inhibition against both Gram-positive and Gram-negative bacteria, due to the presence of phytochemicals such as alkaloids, glycosides, terpenoids etcetera which were observed as present in phytochemical screening. As in this experiment *Blumea lacera* (Burm.f.) DC. showed greater antibacterial efficiency, evidenced by it's larger zones of inhibition compared to *Catharanthus roseus* (L.) G. Don., positioned it as a promising candidate in novel antibacterial drug development. These results emphasize the importance of thoroughly exploring and standardizing traditional medicinal plant like *Blumea lacera* (Burm.f.) DC. to ensure consistent and safe therapeutic

applications. Overall, this study contributes significantly to phytomedicine by confirming the more antibacterial promise of *Blumea lacera* (Burm.f.) DC. with respect to *Catharanthus roseus* (L.) G. Don..

METHODS

Aim

This study is aimed to compare the traditional and modern methods of extraction of whole plant of *Blumea lacera* (Burm.f.) DC. to detect the suitable technique to extract specific phytoconstituents and detect it's *in-vitro* antibacterial effect of whole plant extract with respect to *Catharanthus roseus* (L.) G. Don. which will be effective in the development of new era of antibacterial medicines.

Design and setting of the study

All required chemicals and glass apparatus were used in this experiment from Pharmacognosy and Microbiology Laboratory of PG Institute of Medical Sciences,Chandrakona Town, West Midnapore, West Bengal.

Sample collection

Blumea lacera(Burm.f.) DC.often known as Blumea or "Blumea plants", belong to the family Asteraceae. These plants are commonly found in tropical and subtropical regions including roadsides and disturbed lands. Here the source of *Blumea lacera* (Burm.f.) DC.was roadside environment of Chandrokona Town, West Midnapore, West Bengal . *Catharanthus roseus* (L.) G. Don also known as Periwinkle, belong to the family Apocynaceae. These plants are commonly found in roadsides, in shaded woods, and in disturbed, well-drained ground. Here the source of *Catharanthus roseus* (L.) G. Don was the herbal garden of PG Institute of Medical Sciences, Chandrokona Town, West Midnapore, West Bengal . The authentication was proceeded by the help of Botanical Garden, Shibpur, Howrah. During authentication *Blumea lacera* (Burm.f.) DC. was considered under Specimen number PGIMS/BBK-01 and *Catharanthus roseus* (L.) G. was considered under Specimen number PGIMS/BBK-02.

Extraction procedure:

- At first collect the whole plant of *Blumea lacera* (Burm.f.) DC. and *Catharanthus roseus* (L.) G..
- Thoroughly washed the whole plant with tap water and then with distilled water to remove all type of dirts and impurities.
- After cleaning all parts of the plants were cut into small pieces by used a sharp knife.
- Then dried the whole plant into a hot air oven under 60 degree centigrade constant temperature for 24 hours [13].
- After that completely dried plant were crushed by used a mortar pestle to produce a fine powder and proceeded for extraction.

Cold maceration [14]

- Took 40 gram of dried powder of *Blumea lacera* (Burm.f.) DC.in a round bottom flask and proceeded for the method of cold maceration for 24 hours where 400 ml of methanol was used as solvent.
- After 24 hours filtered the mixture and collected the filtrate.
- The marc were again proceeded for extraction as previous and repeated the process for 5 days until the menstruum became colourless.
- After completion of the cold maceration process, combined the filtrate and proceeded for the solvent evaporation method at below 50 degree centigrade temperature.

- As a result collected a viscous semisolid mass.
- The collected residue were kept in a desiccator for further study.

Ultrasound assisted extraction [15]

- Took 40 gram of dried powder of *Blumea lacera* (Burm.f.) DC.in a conical flask and proceeded for Ultrasound Assisted Extraction for 30 minutes where methanol was used as solvent for extraction.
- After successful extraction the mixture was filtered and collected the filtrate.
- Then proceeded for the solvent evaporation method at below 50 degree centigrade temperature.
- As a result collected a viscous semisolid mass.
- The collected residue were kept in a desiccator for further study.

Soxhlet extraction [16]

- Took 40 gram of dried powder of *Blumea lacera* (Burm.f.) DC. and *Catharanthus roseus* (L.) G. separately into two thimbles and the thimbles were kept into the soxhlet chamber for extraction at below 50 degree centigrade temperature where methanol was used as solvent for extraction.
- After 8 hours of successful extraction the solvent was proceeded for the solvent evaporation method at below 50 degree centigrade temperature.
- As a result collected a viscous semisolid mass.
- The collected residue were dried in a desiccator for further study.

Qualitative Analysis of *Blumea lacera* (Burm.f.) DC.:

Table 6: Phytochemical screening of different extract of *Blumealacera* whole plant [17]

SL NO	PHYTOCONSTITUTES	TEST PROCEDURE	OBSERVATION		
			COLD MACERATION EXTRACT	ULTRASOUND ASSISTED EXTRACT	SOXHLATION EXTRACT
1.	ALKALOIDS	**Dragendorff's test**	Raddish brown precipitate was found	Raddish brown precipitate was found	Raddish brown precipitate was found
		Mayer's test	No colour was found	No colour was found	No colour was found
		Wagner's test	Raddish brown colour was found	Raddish brown colour was found	Raddish brown colour was found
		Hager's test	Yellow colour was found	Yellow colour was found	Yellow colour was found
2.	CARBOHYDRATES	**Molisch's test**	No ring was found	Violet ring was found	Violet ring was found
		Barfoed's reagent	No colour was found	Brick red precipitate was found	No colour was found
3.	TANNINS	**Ferric chloride test**	No colour was found	No colour was found	No colour was found
4.	STARCH	**Iodine test**	No colour was found	No colour was found	No colour was found
5.	STEROIDS AND TRITERPENOIDS	**Liebermann-Burchard test**	No ring was found	Brown ring was formed	Brown ring was formed
		Salkowski test	No ring wasfound	Red colour ring was formed	no ring was found
6.	CARDIAC GLYCOSIDES	**Baljet's test**	Orange colour formed	Orange colour formed	Orange colour formed
		Legal's test	No colour found	Blood red colour found	Blood red colour found
7.	PROTEINS	**Xanthoproteic test**	No colour found	Yellow colour formed	No colour found
		Biuret test	No colour found	No colour found	No colour found

Study of Comparative Antibacterial Activity of Soxhlate Extract [18] **:**

Here crock borer method was used to determine the antibacterial activity of Soxhlate extract of whole plant of *Blumea lacera* (Burm. f.) DC. with respect to whole plant of *Catharanthus roseus* (L.) G. Don.. Here the antibiotic assay media number 1 containing petridishes were aseptically inoculated with *Escherichia Coli* (ATCC-8739), *Staphylococcus aureus* (ATCC-6538), *Salmonella abony* (NCTC-6017) and *Pseudomonas aeruginosa* (ATCC-9027) separately. Then 100 micro litters of Oxytetracycline Hydrochloride (Standard) with concentrations 100 mg/litre which was considered as higher dose (H) and 50 mg/litre which was considered as lower dose (L)were respectively inoculated aseptically into the left hand side bores of culture media. In the same manner 100 µlt of whole plant extract of *Blumea lacera* (Burm. f.)

DC. and *Catharanthus roseus* (L.) G. Don.with concentrations 100 mg/litre which was considered as higher dose (H) and 50 mg/litre which was considered as lower dose (L)were respectively inoculated aseptically into the right hand side bores of culture media into separate petridishes. In all dilution of standard and sample, methanol was used as solvent.After successful inoculation the petridishes were incubated at 37°C for 5 days. The sensitivity of a particular strain to as specific extract is determined by the diameter of the inhibited zone in the petridish in respect to blank, was recorded.

Abbreviations

mg
Milligram
µlt
Micro litter
℃
Degree centigrade

References

1. MASYUDI M, Hanafiah M, RINIDAR R, Usman S, MARLINA M (2022) Phytochemical screening and GC-MS analysis of bioactive compounds of Blumea balsamifera leaf extracts from South Aceh, Indonesia. Biodiversitas Journal of Biological Diversity 23(3)
2. Van Van D, Tra N. T, Le Ngoc Quynh Giao D.A, Son N. V. N. PHYTOCHEMICAL INVESTIGATION AND ATIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF Blumea densiflora var. hookeri (CB Clarke ex Hook. f.) CC Chang & YQ Tseng LEAVES.
3. Rao A. M (2021) In vitro antioxidant and anticancer activity of Blumea lacera leaf extract. Journal of Biotech Research 12
4. Khair A, Ibrahim M, Ahsan Q, Homa Z, Kuddus M. R, Rashid R. B, Rashid M. A (2014) Pharmacological activities of Blumea lacera (Burm. f) DC: a medicinal plant of Bangladesh. British Journal of Pharmaceutical Research 4(13) : 1677
5. Khandekar U, Tippat S, Hongade R (2013) Investigation on antioxidant, anti-microbial and phytochemical profile of Blumea lacera leaf. Int J Biol Pharm Res 4(11) : 756-761
6. Patil P. J, Ghosh J. S (2010) Antimicrobial activity of Catharanthus roseus–a detailed study. British Journal of Pharmacology and Toxicology 1(1) : 40-44
7. Aslam J, Khan S. H, Siddiqui Z. H, Fatima Z, Maqsood M, Bhat M. A, Sharma M. P (2010) Catharanthus roseus (L.) G. Don. An important drug: it's applications and production. Pharmacie Globale (IJCP) 4(12): 1-16
8. Govindasamy C, Srinivasan R (2012) In vitro antibacterial activity and phytochemical analysis of Catharanthus roseus (Linn.) G. Don. Asian Pacific Journal of Tropical Biomedicine 2(1): S155-S158
9. Reichling J (2010) Plant-microbe interactions and secondary metabolites with antibacterial, antifungal and antiviral properties. Annual plant reviews volume 39: Functions and biotechnology of plant secondary metabolites 39: 214-347
10. Felix G, Soto-Robles C. A, Nava E, Lugo-Medina E (2021) Principal metabolites in extracts of different plants responsible for antibacterial effects. Chemical Research in Toxicology 34(9) : 1970-1983
11. Shrivastav G, Prava Jyoti T, Chandel S, Singh R (2024) Eco-friendly extraction: innovations, principles, and comparison with traditional methods. Separation & Purification Reviews 1-17

12. Nortjie E, Basitere M, Moyo D, Nyamukamba P (2022) Extraction methods, quantitative and qualitative phytochemical screening of medicinal plants for antimicrobial textiles: a review. Plants 11(15): 2011
13. Altemimi A, Lakhssassi N, Baharlouei A, Watson D. G, Lightfoot D. A (2017) Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants 6(4) : 42
14. Aleixandre-Tudo J. L, du Toit W (2018) Cold maceration application in red wine production and its effects on phenolic compounds: A review. Lwt 95: 200-208
15. Tiwari B. K (2015) Ultrasound: A clean, green extraction technology. TrAC Trends in Analytical Chemistry 71: 100-109
16. López-Bascón M. A, De Castro M. L (2020) Soxhlet extraction. In Liquid-phase extraction. Elsevier pp. 327-354
17. Shaikh J. R, Patil M (2020) Qualitative tests for preliminary phytochemical screening: An overview. International journal of chemical studies 8(2): 603-608
18. Eloff, J. N (1998) A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta medica 64(08): 711-713

Figures

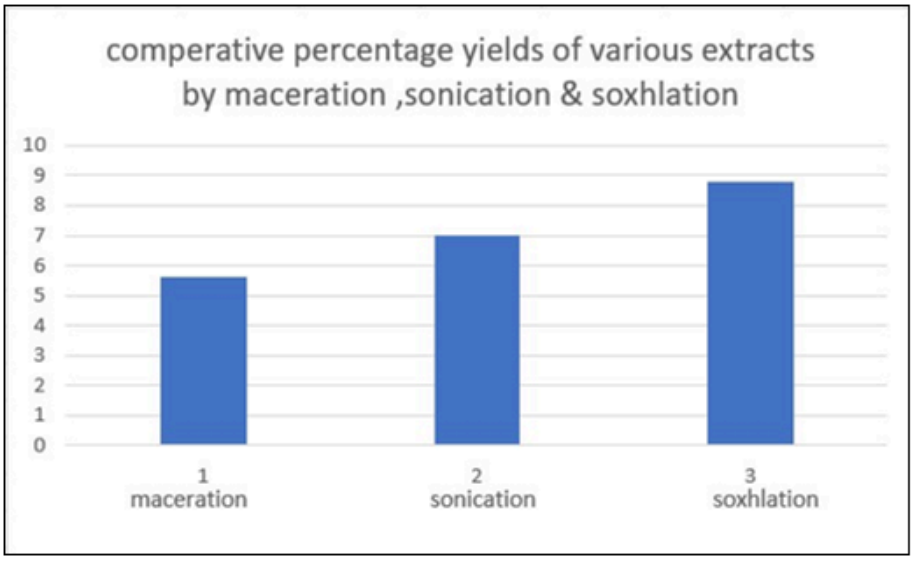

Figure 1

Comperative percentage yields of methanolic extract of whole plant of *Blumea lacera* (Burm.f.) DC by cold maceration, ultrasound sonication & soxhlation

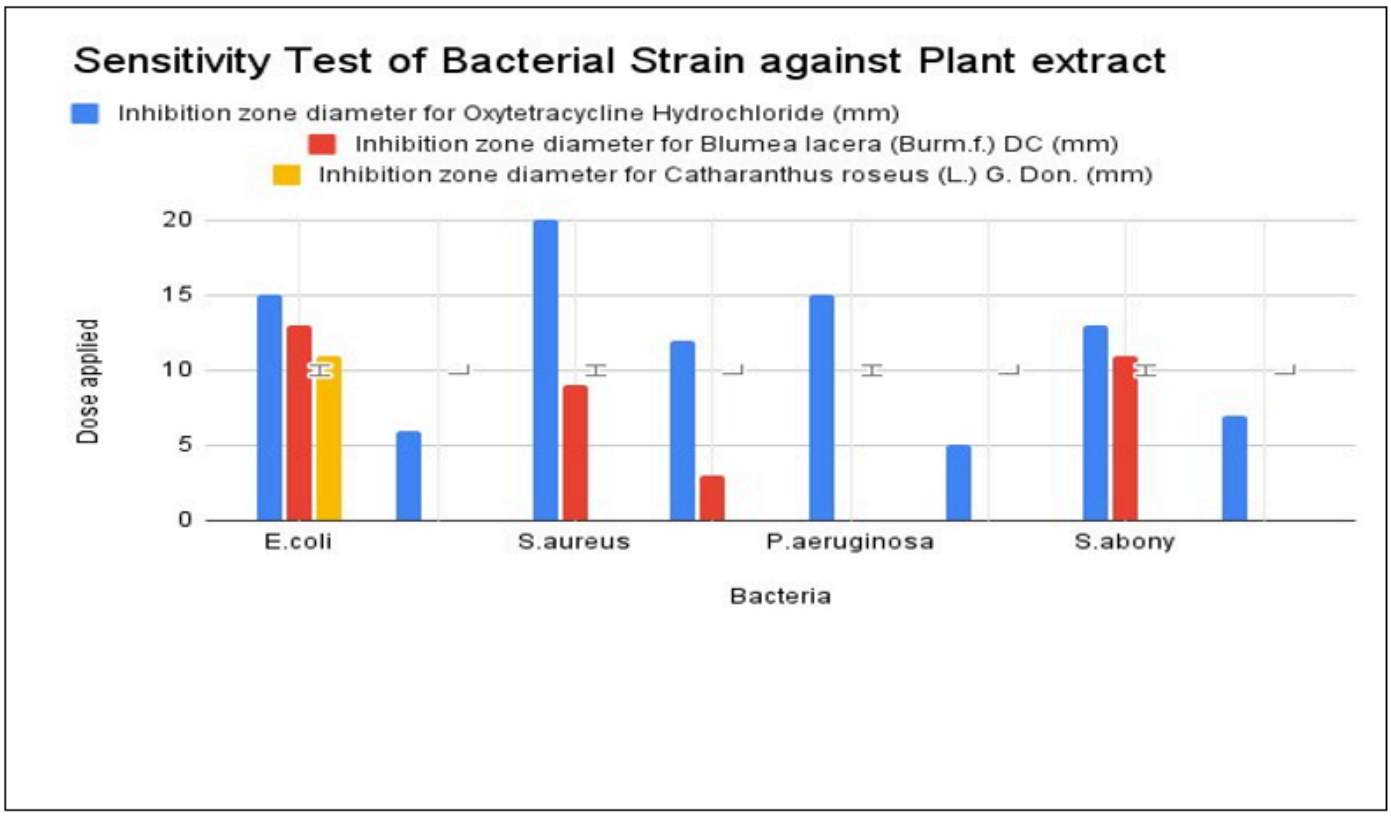

Figure 2

Sensitivity Test of Bacterial Strain against Plant extract

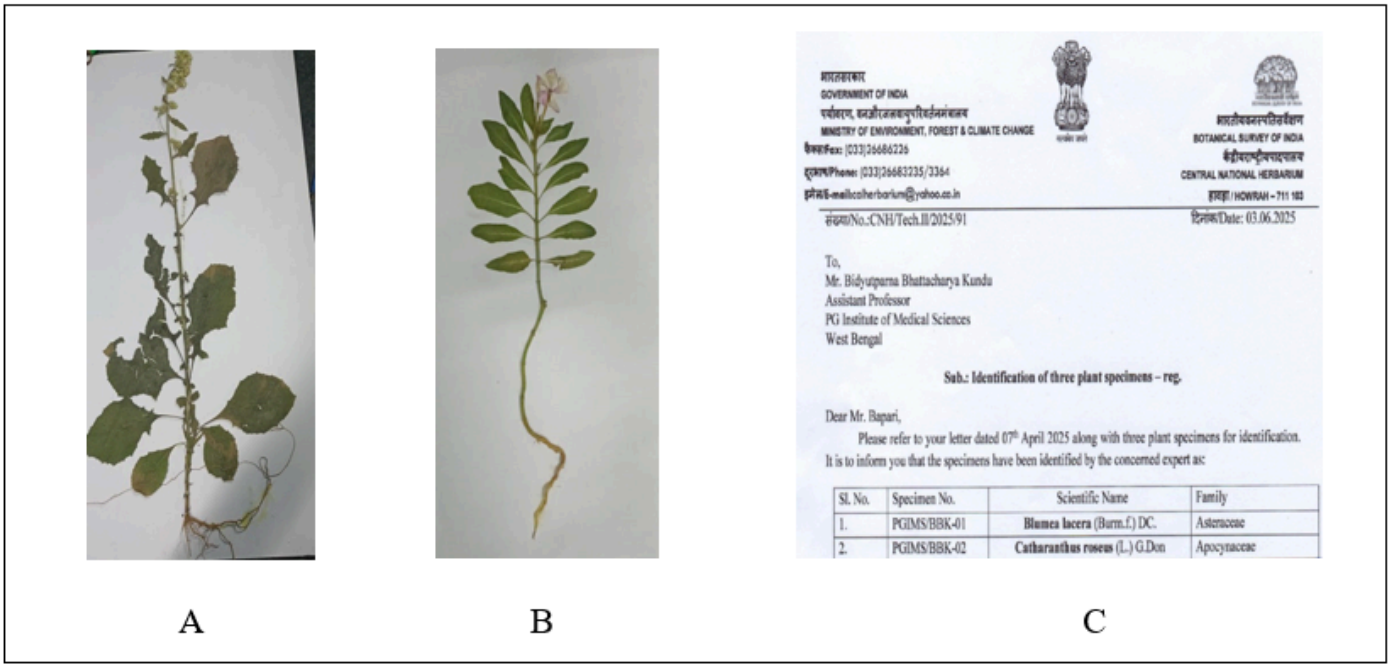

Figure 3

Authentication of Plants [A: Herbarium of *Blumea lacera* (Burm.f.) DC.; B: Herbarium of *Catharanthus roseus* (L.) G. Don.; C: Authentication report from BOTANICAL SURVEY OF INDIA]

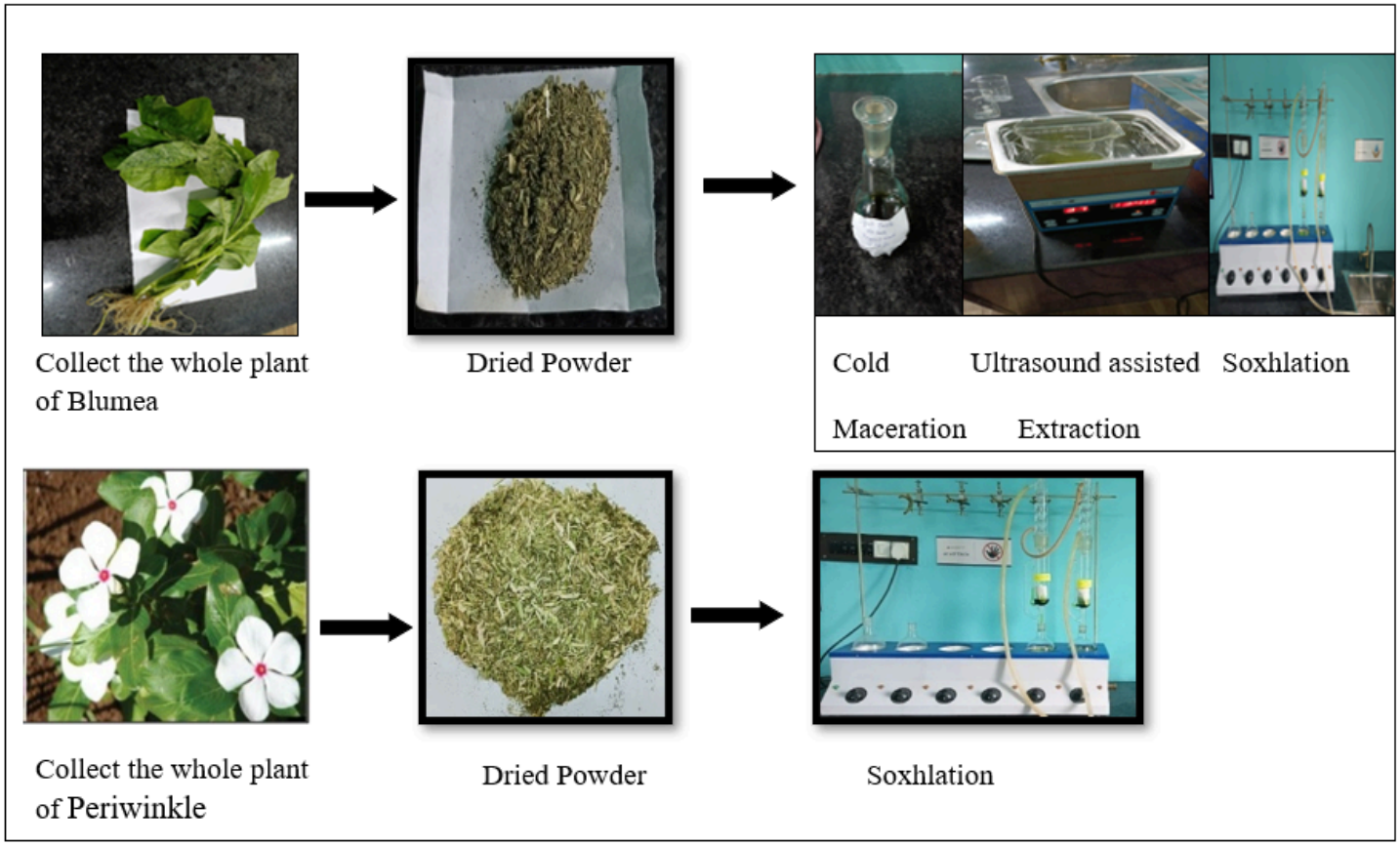

Figure 4

Steps of Extraction

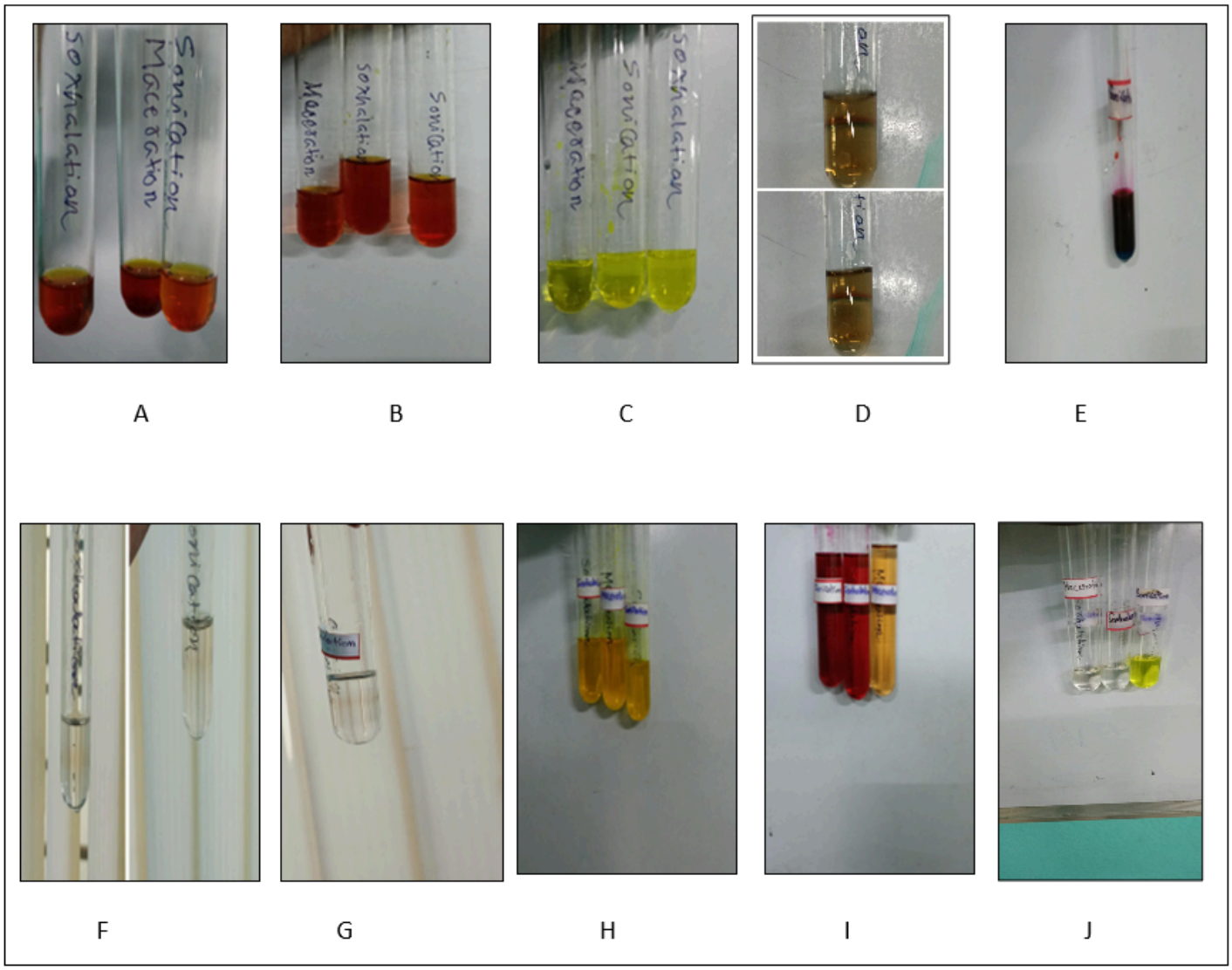

Figure 5

Observations of phytochemical screening of cold maceration, ultrasound assisted extraction and soxhlation derived extracts separately [A: Dragendorff test; B: Wagner's test; C: Hager's test; D: Molisch test with soxhlate and ultrasound assisted extract; E: Barfoed test with ultrasound assisted extract; F: Libermann-Burchard test with soxhlate and ultrasound assisted extract; G: Salkowski test with ultrasound assisted extract; H: Baljet's test; I: Legal's test with soxhlate and ultrasound assisted extract; J: Xanthoproteic test with ultrasound assisted extract.]

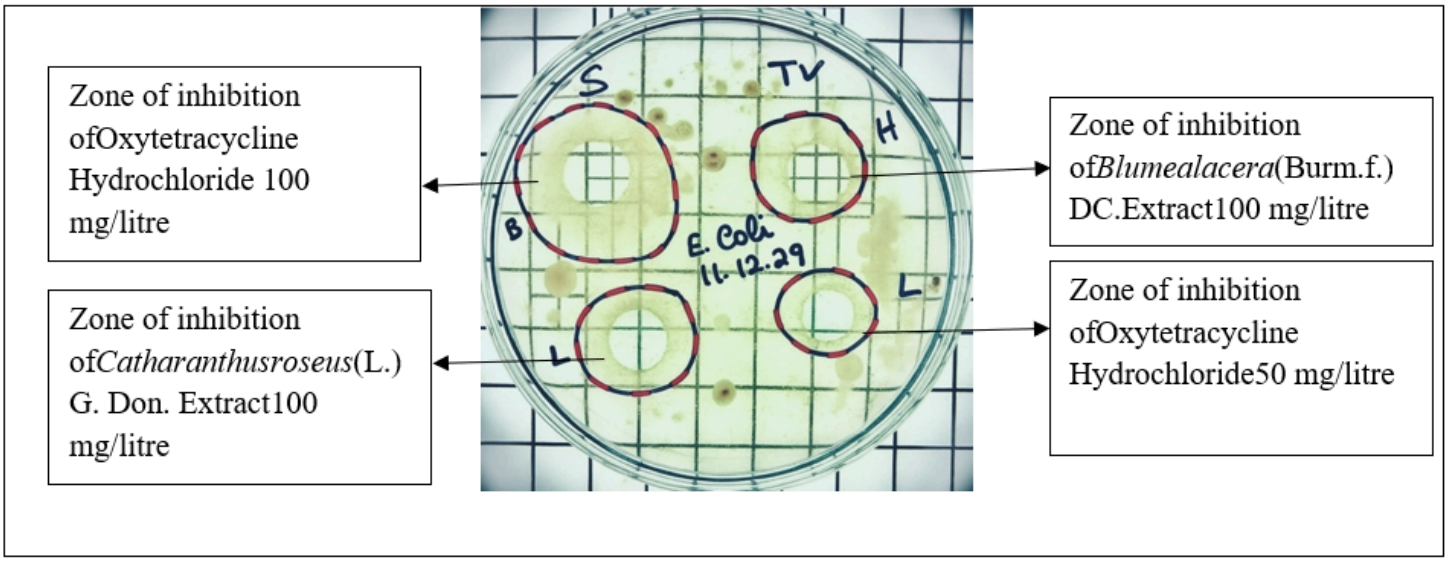

Figure 6

Observations of Comparative Antibacterial Effect against *Escherichia Coli* (ATCC-8739)