EVALUATION OF PRESERVATIVE PROPERTIES IN PSIDIUM GUAJAVA LEAFS USING HYDRO-ALCOHOLIC EXTRACTION

Presented By

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Menu

End

General Description

- A preservative is a natural or synthetic chemical that is added to products such as foods, pharmaceuticals, paints, biological samples, etc.
- To retard spoilage, whether from microbial growth, or undesirable chemical changes.
- Typically copper, borate, and petroleum based chemical compounds are used

Menu

Chemical Preservatives

- SODIUM PROPIONATE
- PROPYL GALLATE
- SULFITES
- PARABENS
- QUATERNIUM-15

Menu

SODIUM PROPIONATE

- Preservative*: Bread, rolls, pies, cakes.*
- Prevents mold growth on bread and rolls.
- A beneficial mineral; the propionate is safe. Sodium propionate is used in pies and cakes, because calcium alters the action of chemical leavening agents.

Chemical Preservatives

PROPYL GALLATE

- Antioxidant preservative: *Vegetable oil, meat products, potato sticks, chicken soup base, chewing gum*.
- Retards the spoilage of fats and oils and is often used with BHA and BHT, because of the synergistic effects these preservatives have.
- The best studies on rats and mice were peppered with suggestions that this preservative might cause cancer.

SULFITES (SULFUR DIOXIDE, SODIUM BISULFITE)

- Prevent discoloration (dried fruit, some "fresh" shrimp, and some dried, fried, or frozen potatoes) and bacterial growth (wine).
- Destroy vitamin B-1
- Can cause severe reactions, especially in asthmatics.

METHYL, PROPYL, BUYTL, and ETHYL PARABEN

- Used as preservatives to inhibit microbial growth to extend products shelf life.
- May cause allergic reactions and rashes.
- Contain highly toxic formaldehyde-releasing ingredients which are carcinogenic, increasing the risk of cancer in both men and women.

Quaternium-15

- Used as a preservative in cosmetics and toiletry items, as well as skin moisturizers and hair care products.
- It commonly causes allergic reactions and dermatitis, and breaks down into formaldehyde

Chemical Preservatives

Natural Preservatives

- Grape Fruit Seed Extract
- Alpha Tocopherol
- Potassium Sorbate
- Citric Acid

Menu

Grapefruit Seed Extract (gse)

- An all natural preservative -- there are rumors that grapefruit seed extract works as a natural preservative.
- There was a study done on the preservative qualities of gse. This study found that it had been contaminated with triclosan and other chemical preservatives. The study also took a handmade grapefruit seed extract that was not tainted with other chemicals.
- Causes candidiasis, earache, throat infections, and diarrhea.

Natural Preservatives

ALPHA TOCOPHEROL
(Vitamin E)

- Antioxidant, nutrient: *Vegetable oil*. Vitamin E is abundant in whole wheat, rice germ, and vegetable oils. It is destroyed by the refining and bleaching of flour.
- Vitamin E prevents oils from going rancid.
- Recent studies indicate that large amounts of vitamin E may help reduce the risk of heart disease and cancer.

Potassium Sorbate

- Effectively preserve against mold and yeast, it is not useful for protecting the product from bacteria.
- It is also not effective at all in products with a pH over 6, which most lotions are. While potassium sorbate is found in nature, any available today would have been synthetically made so it is not all-natural.
- It is also believed to cause contact dermatitis.

CITRIC ACID

- Acid, flavoring, chelating agent: *Ice cream, sherbet, fruit drink, candy, carbonated beverages, instant potatoes*.
- Citric acid is versatile, widely used, cheap, and safe. It is an important metabolite in virtually all living organisms and is especially abundant naturally in citrus fruits and berries.
- It is used as a strong acid, a tart flavoring, and an antioxidant.

Why,

Natural Preservatives ?

- Synthetic preservatives are mostly being the source of life threatening disease – cancer, dermatitis, blindness.
- Natural preservatives- derived from mother nature and easily accustomed by human body.

Menu

Study Objective

- To provide an alternative option toward healthier and safer product preservation against microorganism and fungal habitation in food and neutraceutical products by using natural resources.

Menu

Studies Conducted On Potential Plants

- Antibacterial potential from Indian *Suregada angustifolia*.
- Antimicrobial activity of the methanolic extract from the stem bark of *tridesmostemon omphalocarpoides* (*Sapotaceae*).
- Phytochemical and antimicrobial studies of *Begonia malabarica*.

Menu

Table 2
Inhibition zones[a] (mm) for the methanolic extract, fractions of *Tridesmostemon omphalocarpoides* and reference antibiotics

Tested sample[b]	Bacteria						Fungi[b]		
	Escherichia coli	*Proteus vulgaris*	*Shigella dysenteriae*	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Streptococcus faecalis*	*Salmonella typhi*	*Candida albicans*	*Candida krusei*
ME	28.0 ± 0.0	16 ± 0.0	17.0 ± 0.0	24.0 ± 0.0	16 ± 0.0	24.0 ± 0.0	25.0 ± 0.0	21 ± 0.0	23 ± 0.0
TOHex	20.33 ± 2.52	18.0 ± 0.0	15.0 ± 0.0	13.33 ± 0.57	17.0 ± 0.0	26.0 ± 0.0	22.0 ± 0.0	17.0 ± 1.0	17.0 ± 0.0
TOHex1	20.0 ± 0.0	18.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	24.33 ± 0.57	29.0 ± 0.0	20.33 ± 2.52	20.0 ± 0.0	19.0 ± 0.0
TOHex2	16.0 ± 0.0	20.0 ± 0.0	12.0 ± 0.0	6.0 ± 0.0	30.0 ± 2.0	31.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0	23.0 ± 0.0
TOHex3	18.0 ± 0.0	18.0 ± 0.0	17.0 ± 0.0	9.0 ± 0.0	15.0 ± 0.0	28.0 ± 1.0	12.0 ± 0.0	23.0 ± 0.0	23.0 ± 0.0
TOHex4	18.0 ± 0.0	14.0 ± 0.0	17.0 ± 0.0	8.0 ± 0.0	18.0 ± 0.0	27.0 ± 0.0	12.0 ± 0.0	20.33 ± 2.52	18.0 ± 0.0
TOHex5	19.0 ± 0.0	15.0 ± 0.0	15.0 ± 0.0	16.0 ± 0.0	20.0 ± 0.0	24.0 ± 0.0	14.0 ± 0.0	20.0 ± 0.0	18.0 ± 0.0
TOAc	28.0 ± 1.0	17.0 ± 0.0	22.0 ± 0.0	21.0 ± 0.0	23.0 ± 0.0	23.0 ± 0.0	21.0 ± 0.0	23.0 ± 0.0	23.33 ± 0.57
TOAc1	14.0 ± 0.0	13.0 ± 0.0	22.0 ± 0.0	18.0 ± 0.0	20.0 ± 0.0	21.0 ± 0.0	16.0 ± 0.0	20.0 ± 0.0	18.0 ± 0.0
TOAc2	20.0 ± 0.0	18.0 ± 0.0	19.0 ± 0.0	20.0 ± 0.0	20.0 ± 1.0	18.0 ± 0.0	14.0 ± 0.0	14.0 ± 0.0	14.0 ± 0.0
TOAc3	6.0 ± 0.0	6.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	14.0 ± 0.0	14.0 ± 0.0
TOAc4	8.0 ± 0.0	6.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	6.0 ± 0.0	10.0 ± 0.0	8.0 ± 0.0	30.0 ± 0.0	33.0 ± 0.0
TOAc5	18.0 ± 0.0	18.0 ± 0.0	19.0 ± 0.0	21.0 ± 0.0	24.0 ± 0.0	20.0 ± 0.0	29.0 ± 0.0	28.0 ± 1.0	31.0 ± 0.0
TOAc6	20.0 ± 0.0	18.0 ± 0.0	21.0 ± 0.0	19.0 ± 0.0	20.0 ± 0.0	18.0 ± 0.0	30.66 ± 1.25	20.0 ± 0.0	18.0 ± 0.0
TOAc7	17.0 ± 0.0	13.0 ± 0.0	18.0 ± 0.0	17.0 ± 0.0	12.0 ± 0.0	15.0 ± 0.0	22.0 ± 0.0	18.0 ± 0.0	21.0 ± 0.0
TORe	17.0 ± 0.0	15.0 ± 0.0	19.0 ± 0.0	26.0 ± 0.0	23.0 ± 0.0	18.0 ± 0.0	6.0 ± 0.0	15.0 ± 0.0	17.0 ± 0.0
TORe1	14.0 ± 1.0	11.0 ± 0.0	13.0 ± 0.0	17.0 ± 0.0	19.0 ± 0.0	12.0 ± 0.0	6.0 ± 0.0	9.0 ± 0.0	16.0 ± 0.0
TORe2	13.0 ± 0.0	13.33 ± 0.57	16.0 ± 0.0	19.0 ± 0.0	19.0 ± 0.0	15.0 ± 0.0	6.0 ± 0.0	13.0 ± 0.0	16.0 ± 0.0
TORe3	17.0 ± 0.0	19.0 ± 0.0	18.0 ± 0.0	24.33 ± 0.57	21.0 ± 0.0	19.0 ± 0.0	6.0 ± 0.0	15.0 ± 0.0	19.33 ± 0.57
TORe4	20.33 ± 0.57	21.66 ± 1.25	20.0 ± 0.0	28.33 ± 2.52	23.0 ± 0.0	22.33 ± 0.57	6.0 ± 0.0	17.33 ± 0.57	21.0 ± 0.0
TORe5	19.0 ± 1.0	19.33 ± 0.57	20.33 ± 0.57	19.0 ± 0.0	23.33 ± 0.57	17.0 ± 0.0	6.0 ± 0.0	17.0 ± 0.0	19.66 ± 1.25
TORe6	11.0 ± 0.0	14.0 ± 0.0	13.0 ± 0.0	16.0 ± 0.0	17.0 ± 0.0	14.0 ± 0.0	6.0 ± 0.0	10.0 ± 0.0	16.0 ± 1.0
TORe7	8.0 ± 0.0	8.0 ± 0.0	10.0 ± 0.0	11.0 ± 0.0	8.0 ± 0.0	10.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	10.0 ± 0.0
TORe8	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0
RA[c]	20.0 ± 0.0	24.33 ± 0.57	21.0 ± 0.0	22.0 ± 0.0	19.0 ± 0.0	24.0 ± 0.0	24. 0 ± 0.0	22.0 ± 0.0	22.0 ± 0.0

[a] Including the diameter of the hole (6 mm).
[b] Tested samples (sample tested at 80 mg/ml for ME, 40 mg/ml for TOHex, TOAc and TORe, 10 mg/ml TOHex1-5 and TOAc1-7, 2 mg/ml for RA, no effect of the Tween 80 and DMSO 10% used as dilution solvent was observed on the tested microbial strains.
[c] RA: reference antibiotics (Gentamycin for bacteria, Nystatin for yeast).

Source : Antimicrobial activity of the methanolic extract from the stem bark of tridesmostemon omphalocarpoides (Sapotaceae)
V. Kuete a, J.G. Tangmouob, V. Penlap Benga,, F.N. Ngounoub, D. Lontsi

Studies Conducted

Table 1
Antibacterial activity of aqueous and solvent extracts from the leaves of *Suregada angustifolia*

Microorganisms	Methanol extract (mg/ml)			Chloroform extract (mg/ml)			Hexane extract (mg/ml)			Standard
	20	10	5	20	10	5	20	10	5	
Aeromonas hydrophila	15	12	10	–	–	–	20	16	14	20 (Tr)
Enterobacter aerogenes	24	18	14	–	–	–	18	15	12	22 (Tr)
Escherichia coli	25	20	16	12	10	–	–	–	–	30 (K)
Klebsiella pneumoniae	33	27	22	12	10	–	18	14	12	30 (K)
Proteus vulgaris	30	23	18	16	12	10	14	12	10	20 (K)
Pseudomonas aeruginosa	18	14	12	14	10	–	12	10	–	25 (E)
Salmonella typhi	26	18	14	10	–	–	–	–	–	20 (Na)
Vibrio cholerae	26	19	12	24	18	14	14	12	10	31 (Tr)
Vibrio parahaemolyticus	25	20	15	10	–	–	19	15	12	14 (K)
Vibrio vulnificus	26	20	14	14	10	–	24	18	14	16 (R)
Bacillus subtilis	24	16	12	10	–	–	18	14	11	30 (A)
Staphylococcus aureus	33	27	22	14	10	–	28	21	15	45 (M)

Values (mean of three replicates) are: diameter of zone of inhibition (mm); no inhibition. Ampicillin: A^{10} (10 mcg/disc); erythromycin: E^{10} (10 mcg/disc); kanamycin: K^{30} (30 mcg/disc); methicillin: M^{5} (5 mcg/disc); nalidixic acid: Na^{30} (30 mcg/disc); rifampicin: R^{30} (30 mcg/disc); trimethoprin: Tr^{10} (10 mcg/disc).

Source : Antibacterial potential from Indian *Suregada angustifolia*
M. Venkatesan , M.B. Viswanathan , N. Rameshb, P. Lakshmanaperumalsamy

Studies Conducted

Table 1
Antibacterial activity of aqueous, methanol and chloroform leaf extracts of *Begonia malabarica* (zone of inhibition in mm)

Bacterial strains	Aqueous extract (mg/ml)						Methanol extract (mg/ml)						Chloroform extract (mg/ml)						Standard
	Cold (%)		Boil (%)		Autoclave (%)		50	25	12.5	6.25	3.13	1.56	50	25	12.5	6.25	3.13	1.56	
	100	50	100	50	100	50													
Aeromonas hydrophila	15	–	16	–	18	–	18	18	17	16	15	15	26	25	23	20	17	17	38 (Ce)
Chromobacterium violaceum	28	24	26	24	28	20	23	22	19	18	16	15	27	25	22	22	20	18	24 (Ce)
Escherichia coli	20	16	21	16	20	14	22	20	18	17	17	16	20	18	18	16	15	14	28 (Ch)
Klebsiella pneumoniae	20	14	21	16	20	14	26	25	20	20	18	18	24	24	22	20	18	18	32 (Nv)
Pseudomonas aeruginosa	18	16	20	14	20	16	22	20	18	18	16	16	26	25	22	22	20	17	26 (Ce)
Salmonella typhi	16	14	15	13	14	12	24	22	22	18	16	15	24	22	20	20	19	17	23 (Ce)
Vibrio cholerae	20	16	18	14	18	16	25	23	20	20	17	16	25	23	22	22	19	17	36 (Tr)
Vibrio parahaemolyticus	–	–	–	–	–	–	20	19	18	18	16	16	27	25	24	22	22	18	24 (Er)
Bacillus subtilis	–	–	–	–	–	–	18	17	16	15	12	12	26	24	23	22	18	16	33 (Nv)
Staphylococcus aureus	–	–	–	–	–	–	25	23	21	20	17	17	25	25	20	20	18	16	31 (Nv)

Ce, Ceftriaxone (30 μg/disc); Ch, Chloramphenicol (30 μg/disc); Er, Erythromycin (15 μg/disc); Nv, Novobiocin (30 μg/disc); Tr, Trimethoprim (5 μg/disc).

Source : Phytochemical and antimicrobial studies of *Begonia malabarica*
N. Ramesh , M.B. Viswanathan , A. Saraswathy , K. Balakrishna , P. Brindha ,
P. Lakshmanaperumalsamy

Studies Conducted

Choose,
Psidium guajava (Guava) Leafs

- The extract also showed in vitro antimicrobial activity against *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Proteus mirabilis*, and *Shigella dysenteria* (Iwu,1993).
- Effectiveness of the leaf extract against *Staphylococcus aureus* (Gnan and Demello, 1999).
- It was shown to antibacterial in another study and in addition to *Staphylococcus aureus* was also useful against *Streptococcus spp* (Pranee Jaiarj, et al, 1999).The leaves are rich in tannin, and have antiseptic properties (Hernandez, Dolores F, 1971).

Menu

Utilizing *Psidium guajava* Leaf by

- Manipulating different ethanol-water solvent concentration (95% v/v, 50% v/v and 25% v/v) for *Psidium guajava* leaf extraction via Soxhlet apparatus.
- Analyzing the antibacterial and antifungal properties of *Psidium guajava* leaf extracts on selected microbes and fungal.

Menu

Methodology

- Extraction
- Antimicrobial Test
- Antifungal Test

Extraction Process

- Amount of 25g oven dried leaf powder is extracted with 400ml of 95% ethanol concentrations at 80°C for 18 hours.
- Extract were then filtered with Whatman No.1filter paper before evaporation process.
- Evaporation process was done using rotary evaporator 70°C and 100 mbar.

- Product formed was collected and stored in 4 °C .
- Procedures ware than repeated with 50 % v/v, and 25 % v/v of ethanol concentration as extraction solvent.
- Triplicate every solvent concentration.

Antimicrobial Test

- The bacterial strains used are *Escherichia coli* (gram negative) and *S. aureus* (gram positive).
- Standardized with 0.5 Mc Farland Standard.
- Sample inhibition index was tested using Disc Diffusion Method.
- Inhibition Index =

$$\frac{\text{Inhibition area (hollow zone)}}{\text{Inhibition area of Streptomycin.}}$$

Antifungal Test

- 1ml samples were transferred onto the PDA.
- PDA plates containing 1 ml of 95% ethanol were used as an alcoholic control
- The solid plates are inoculated with 0.1 ml fungal suspension, measuring it into the hole (diameter 8 mm) in the centre of the medium.

- Plates were kept for 10 days, at 28 °C in the dark for germination.
- Inhibition,

$$I = [(C - T)/C] * 100$$

I = inhibition (%).

C = colony diameter of the mycelium on the alcoholic control plate (mm) .

T = colony diameter of the mycelium on the test Petri plate (mm).

Results and Discussions

- Extract Recovery
- Antimicrobial Testing
- Fungal Inhibition Test

Yield of *P.guajava* Leafs Extract

Solvent Concentration (%)	Average Amount of Extract Collected (g)
95	10.096
75	13.605
50	19.026
25	18.893

Physical Appearances of Extract

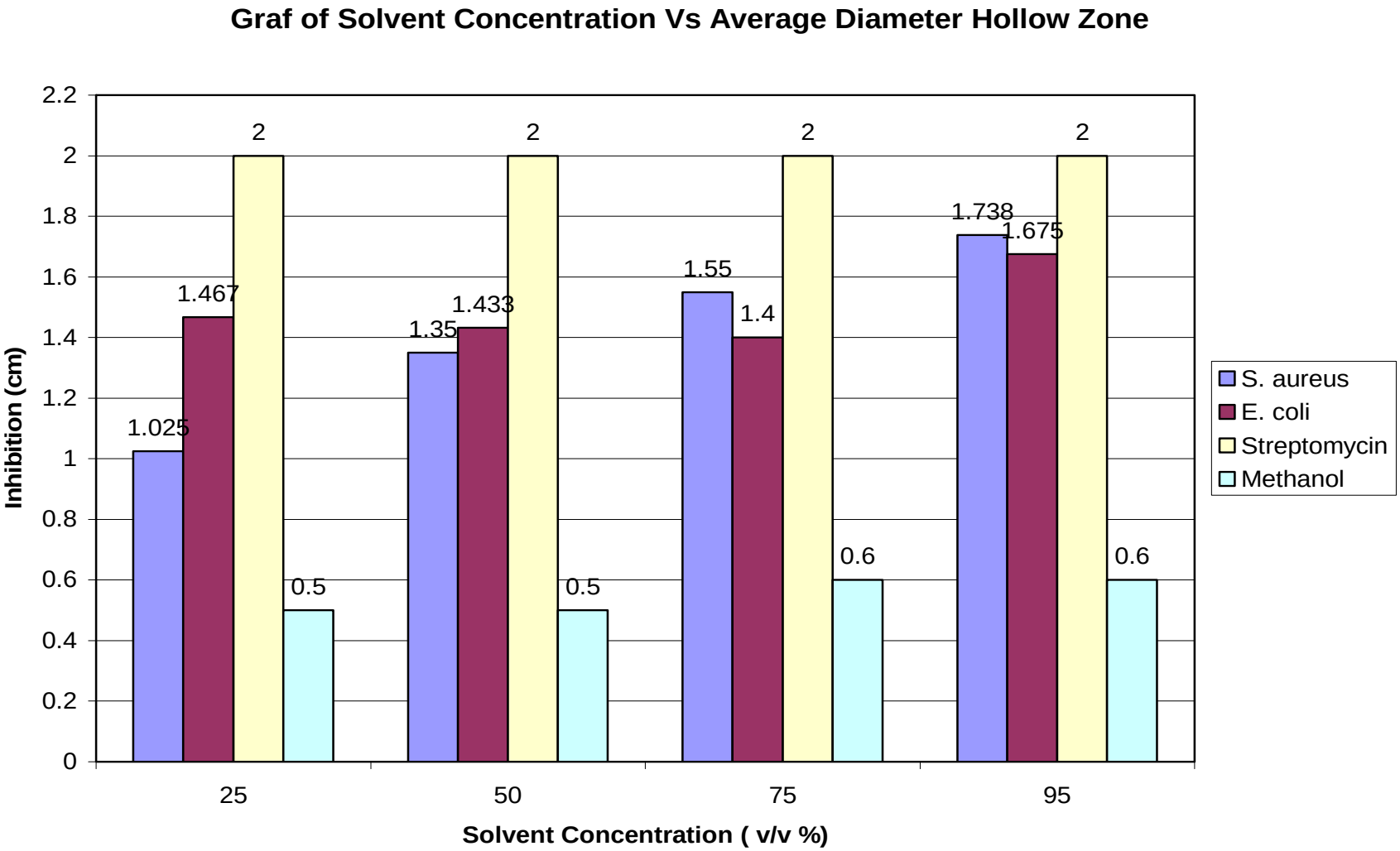
95 % Solvent Extract

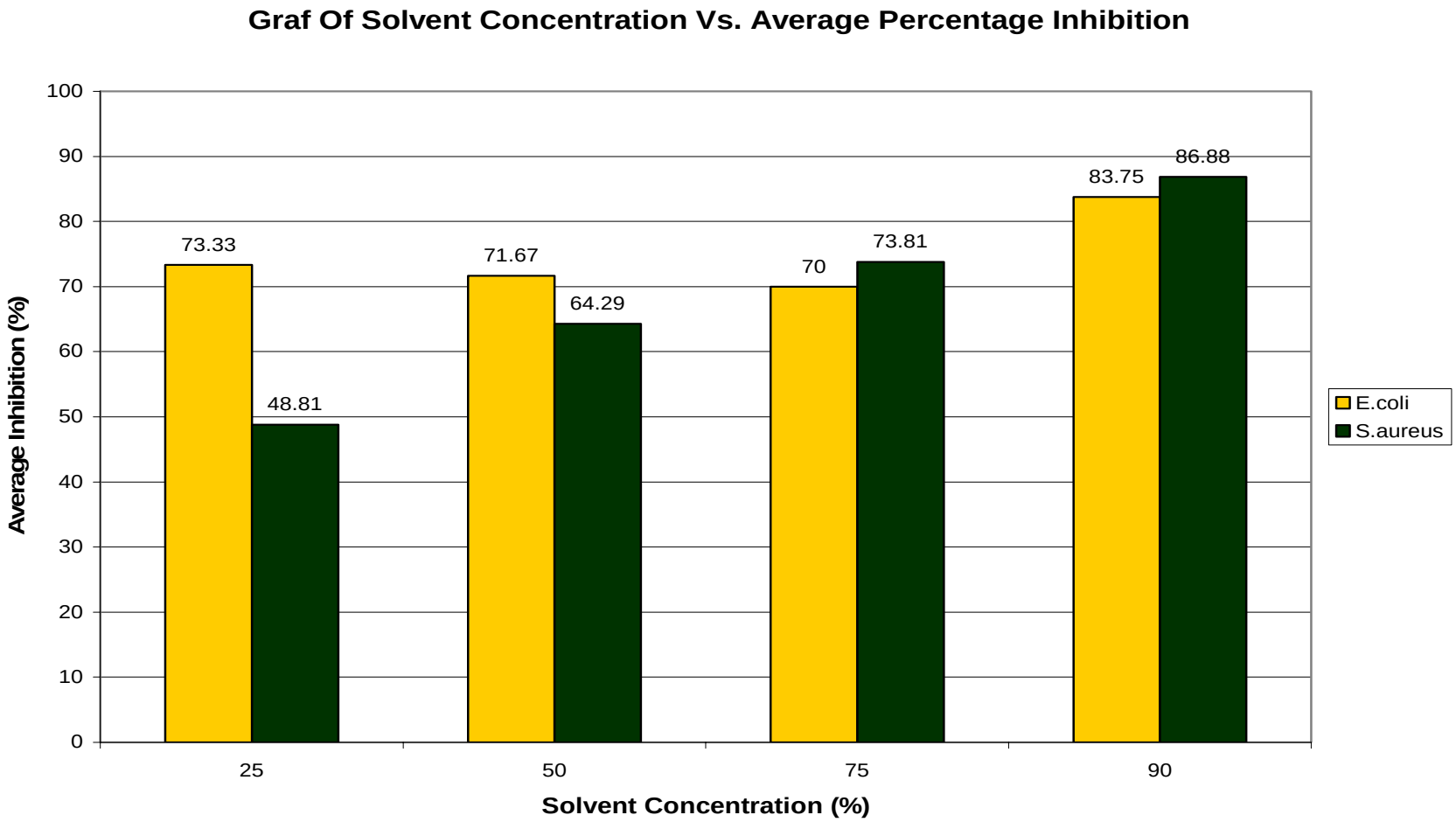
75 % Solvent Extract

50 % Solvent Extract

25 % Solvent Extract

Antimicrobial Testing

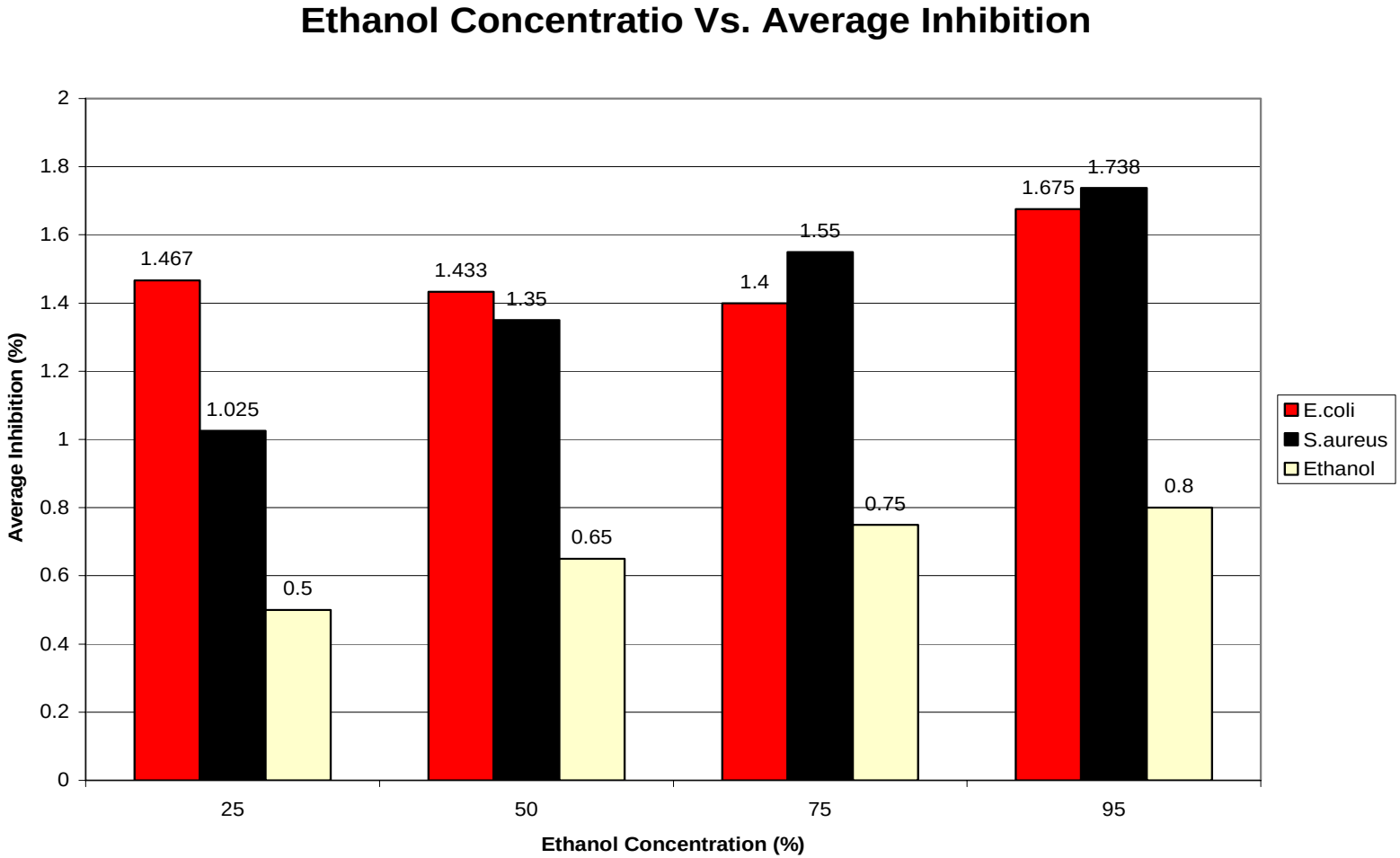



Graf Of Solvent Concentration Vs. Average Percentage Inhibition
Average Inhibition (%)
100
90
80
70
60
50
40
30
20
10
0
73.33
48.81
71.67
64.29
70
73.81
83.75
86.88
25
50
75
90
Solvent Concentration (%)
E.coli
S.aureus

Graph Trending

- Polarity of the solvent.
- Product solubility in solvent and co-solvent. As the purification process commence, bioactive compound were distillate out along with the water-ethanol solvent.
- Main solvent strength reduces via hydrogen bonding before extraction started.

Ethanol ≠ Inhibition Factor



Antifungal Test

Sample	Concentration (%)	Colony Diameter (cm)	Inhibition Effect (%)
1	95	1.2	7.692307692
2	50	1.3	0
3	25	1.3	0
4	95	1.3	0
5	50	1.3	0
6	25	1.3	0
7	75	1.3	0
8	25	1.3	0
9	50	1.3	0
Ethanol	95	1.3	100

Reason Antifungal Failure

- Fungi rapid adaptation to its environment and
- The dilution of ethanol solvent may also contribute to the antifungal failure.

Conclusion

- As a summary for this study, the variation of ethanol-water concentration is most suitable for microbial inhibition rather then fungal inhibition.
- By reducing the amount of ethanol concentration can still inhibit microbes but not 100% inhibition.

Recommendation

- Utilized other extraction technique such as Supercritical Fluid Extraction for better bioactive compound yield.
- Extract samples undergo characterization test.
- Reducing or removing the color intensity and strong odor of extracts for food and neutraceutical products.

Thank You
For The Attention

References

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Next

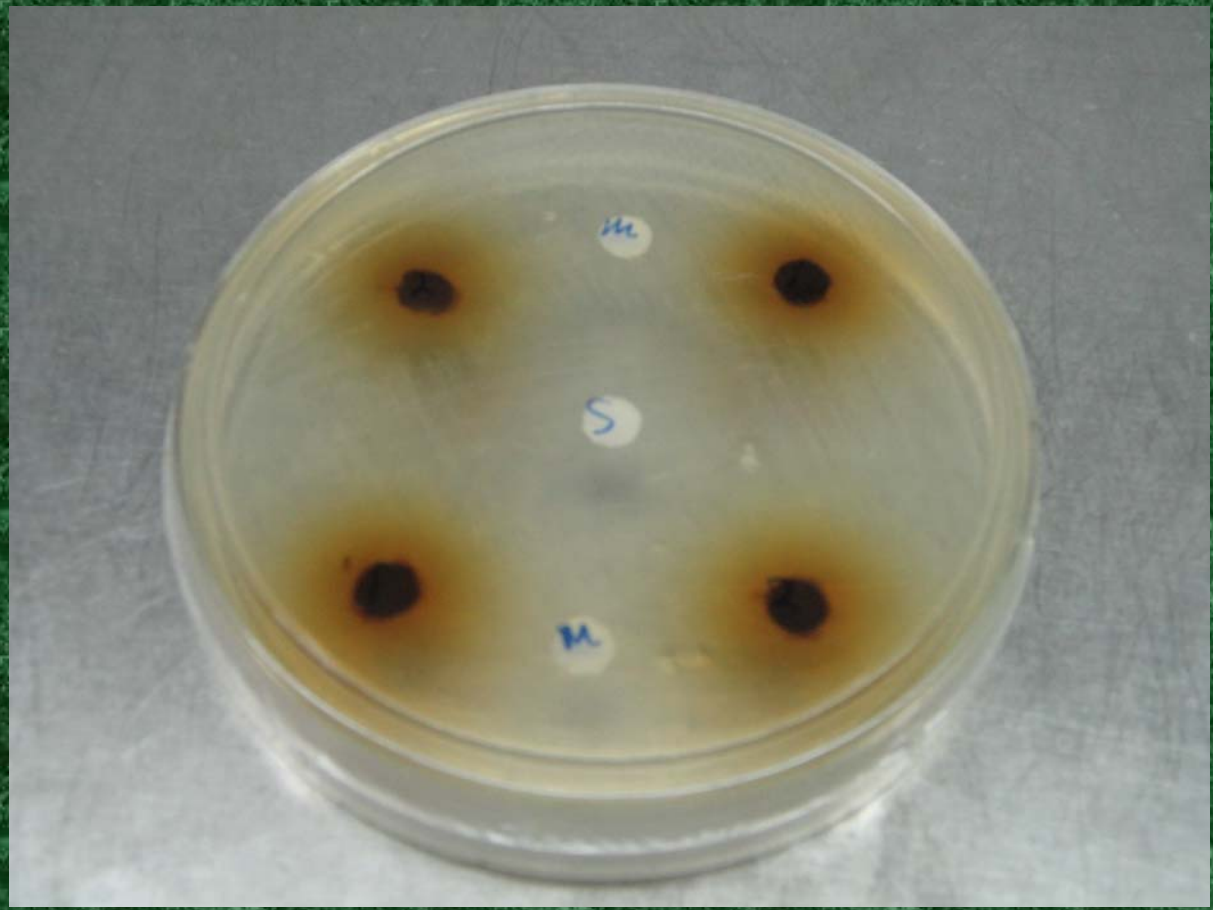
- Phytochemical and antimicrobial studies of *Begonia malabarica*,N. Ramesh , M.B. Viswanathan , A. Saraswathy , K. Balakrishna , P. Brindha ,P. Lakshmanaperumalsamy,20 September 2001.
- Antimicrobial activity of the methanolic extract from the stem bark of tridesmostemon omphalocarpoides (Sapotaceae),V. Kuete a, J.G. Tangmouob, V. Penlap Benga,, F.N. Ngounoub, D. Lontsi, 19 November 2004.
- Antibacterial potential from Indian *Suregada angustifolia*,M. Venkatesan , M.B. Viswanathan , N. Rameshb, P. Lakshmanaperumalsamy, 29 July 2003.

Menu End

Extraction

- Essential oils are usually lipophilic compounds. It thus has been found that alcohols, such as methanol and ethanol (primarily 100% concentrations), or organic solvents, such as acetone, are the best diluents to be used for dilution.
- Water is not recommended as water and fats do not dissolve in one another, although oil dilution in water can be achieved at extremely low concentrations of oil, and depending on the viscosity of the oil.

Menu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